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S I R:

I, Mark Norton, declare that:

1. I am one of the co-inventors of the present application. I have been involved in all stages of the development of the subject matter disclosed in the present application. I am also aware of the pending claims in the present application, as well as the Office Action mailed December 19, 2008.

2. The present invention is directed to a novel discovery by the inventors, namely that consumer liking, i.e. taste preference, for a coffee product can be affected by the addition of a single naturally occurring component in coffee, linalool. The principal consumer sensory characteristics of coffee are its roast quality/bitterness and acidity, as determined by consumer sensory evaluations of numerous diverse coffee types. An extensive independent study was conducted and published in an article by the European Sensory Network Food Research Association, entitled "European Sensory and Consumer Study—A Case Study on Coffee" by J.A. McEwan (relevant excerpts attached in Appendix A). In the study, other attributes were described which play a minor role in characterizing the coffee flavor. Inherent flavors in coffee are described as grassy, green,

citrus, floral, fruity, cereal, roasted, caramel, bitter, woody and winey, as well as other attributes.

3. The flavor of coffee is the result of the coffee beans and the roasting conditions used to produce the roast coffee blend. Depending on the beans and roasting conditions, the coffee blend will be classified by the origin of the coffee bean, e.g., Sumatra, Columbia, Kona, or by roasting conditions, e.g., French roast or Espresso. Although there will be variations in the taste of these blends, collectively they would all be referred to as non-flavored or natural coffee.

4. Coffee itself is a complex product which includes many compounds. Of these flavor compounds, only a few have been previously considered relevant to the taste and aroma in the final coffee beverage. Of the previously considered relevant compounds, they can be classified into groups by the flavor attribute with which they are associated when present within coffee beverages. One example of a flavor group is roasted-nutty.

5. While coffee has natural attributes, in recent years, coffee manufacturers have added an additional flavor to the coffee product, i.e. a non-coffee flavor, which includes hazelnut, chocolate, vanilla, raspberry, Irish crème, toffee, orange, amaretto and marshmallow. The resulting product is referred to as "flavored coffee" to distinguish it from natural coffee product.

6. When processing coffee beans with the intent of positively affecting the intensity of one flavor attribute, commonly there is a negative affect on at least one other flavor attribute. This can be linked to the chemistry of coffee flavor compounds which undergo chemical reactions depending on the roast conditions. Furthermore, due to

different chemical reactions and the roast conditions, it is unpredictable as to what the effect altering even a single flavor, such as adding a flavorant, will have in the final coffee taste.

7. As noted in paragraph 2 above, one naturally occurring component in brewed coffee is linalool. However, prior to the present invention, linalool, was not believed to be of any "major" importance to coffee flavor and/or consumer liking. See, e.g., Blank-I; Sen-A; Grosch-W, "Potent odorants of the roasted powder and brew of Arabica coffee," *Zeitschrift-fuer-Lebensmittel-Untersuchung-und-Forschung*; 195 (3) 239-245, 1992 (hereinafter "Blank et al."), attached to this declaration as Appendix B, which assesses 3,7-dimethylocta-1,6-dien-3-ol, i.e. linalool, as being unimportant in the overall contribution to roasted coffee flavor. The Blank et al. study included 38 compounds that the researchers could detect with their equipment. This included linalool. The summary on the front page highlights the 13 compounds that the group found to be important to the overall perception of coffee – linalool is absent. Further, Ivor Flament, "Coffee Flavour Chemistry," Wiley Press, pp. 104-105 (hereinafter "Flament," attached in Appendix C) suggests it is probably unimportant at the concentrations typically found in brewed coffee. Still, another important reference makes no mention of it within a list of important compounds, namely Illy, A; Viani, R; "Espresso Coffee: The Science of Quality," Elsevier Academic Press, p. 201 (hereinafter "Illy", attached in Appendix D).

8. Linalool is typically present in roast and ground coffee product in an amount between 30 µg/kg and 4700 µg/kg, and in brewed coffee in an amount of 1 µg/l to about 30 µg/l. Linalool, in isolation, is characterized as having a fruity-floral character (see, e.g., Flament, Appendix C).

9. Prior to the present invention, linalool was considered to be an undesirable component in coffee (Flament, pp. 104-15, Appendix C). Specifically, presence of linalool was associated with providing an "undesirable note in disharmony with the notes of a roasted coffee." (*Id.*) Linalool is further described as being a "potent odorant [ ] of roasted powder of Arabica coffee but not in brew." (*Id.*, citing Blank et al.) Further, prior to the present invention, one would not have believed that it would be desirable to add a fruity-floral character in a natural coffee flavor product. Therefore, one did not previously add additional linalool to a coffee product (Flament, Appendix C).

10. Discovery of the present invention proceeded by first taking coffee compounds and identifying their respective contributions to specific sensory coffee attributes in order to assess the importance that each attribute contributes to consumer liking. Prior to the present invention, we were not aware of any other study which determined in detail which groups of compounds were responsible for specific attributes contributing to consumer liking. During our study, we determined how to enhance, degrade or decouple several flavor and aroma attributes by the addition of associated groups of coffee flavor compounds within a robust statistical design. This method permitted us to assess consumer reaction towards enhanced levels of each individual attribute and did not generate unfamiliar attributes within the context of pure coffee. As a result, we were able to "decouple" coffee attributes in order to accurately assess which flavor and aroma attributes contributed to consumer liking. We then determined consumer responses to flavor attributes, i.e. components associated with driving coffee product liking. In addition, we evaluated consumer responses for components that previously had

only been associated with contributing to subtleties of the coffee product profile on a component-by-component basis.

11. Through our experimentation, we surprisingly determined that a single chemical, linalool, which previously was not known to be of primary importance to affect or be associated with sensory attributes, let alone to be important at all in the overall coffee flavor, drove significant consumer liking. Further, it was determined that enhanced consumer liking was attributed to linalool in a roast and ground coffee having levels of linalool, as measured in the roast and ground product, of at least 6000 µg/kg using the Likens method. Based on these results, the amount of linalool in roast and ground coffee, one can extrapolate the data to soluble coffee having levels of at least 2000 µg/kg of soluble coffee solids present in the soluble coffee.

12. Some coffee experts come to regard the delicate Arabica coffee from East Africa as high quality, e.g., Ethopian Sidamo and Ethiopian Djimmah. These origins of coffee are relatively rare and expensive. We have identified that a key differentiating chemical signature within this family of coffee is the higher level of linalool, and found through the course of this work that elevating the level of linalool in more common, lower-linalool variants that we can move the flavor character towards the more expensive and rare Ethiopian coffee.

13. Further, surprisingly we determined that fruity and floral attributes substantially drove consumer liking. Prior to the present determination, it was unknown in the art that fruity and floral attributes drive consumer liking. More importantly, we determined that the fruity and floral attributes of linalool improve the taste of coffee products higher than achievable by varying previously known coffee attributes,

components and traditional drivers. We were surprised by this determination, as one of ordinary skill in the art would not expect a fruity and floral characteristic to enhance the overall liking of traditional, i.e. non-flavored, coffee (see, present specification, paragraphs [0033]-[0040].

14. Moreover, an internal Kraft study and corresponding Kraft Research Report, attached in Appendix F, shows that the presence of linalool resulted in a more desirable coffee flavor over the control coffee.

15. Referring to the determination of a fruity-floral attribute which drove consumer liking in more detail, the determination was conducted by first identifying groups of naturally occurring coffee chemicals, i.e. coffee components, each of which describe a well-known sensory attribute of coffee.

16. In the food art and, in particular, when considering affecting the taste and flavor of a food product, one must be mindful and consider the effect adding one or more flavorant will have on the other compounds or components in the food product to which the flavorant(s) is/are added. As noted above, flavors are often coupled and, in most cases, it is completely unpredictable as to what effect adding one or more flavorant will have on the flavor of the resulting food product. Furthermore, often one does not add just a single flavorant, as typically it will be necessary to add more than one, in order for the resulting food product to have the desired flavor attributes. For example, U.S. Patent No. 4,311,720 (Marmo et al.) discloses a lemon flavored product produced by adding about 21 different ingredients, in order to produce a lemon flavored product.

17. One of ordinary skill in the art would not have been led to add linalool to coffee. Linalool, itself, while known to have a fruity-floral attribute, would not specifically

be described as corresponding to any specific flavor, such as lemon (see above, paragraphs 9, 13 and 14). In fact, linalool is more accurately characterized as "sweet floral, petitgrain-like" (see, e.g., http://en.wikipedia.org/wiki/Linalool, attached in Appendix G). For example, Marmo et al. include it as one of around 21 additional flavor ingredients, in order to produce a lemon flavor. Moreover, one would not describe linalool, itself, as having a lemon flavor. Therefore, should one desire a lemon flavored product, one would not be led to select linalool from among many flavors, which in combination produce a lemon flavor, such as those described in Marmo et al. In order to get a lemon flavor using linalool, one would have to add additional ingredients, as evidenced by Marmo et al.

18. More importantly, prior to our discovery, one of ordinary skill in the art would not have known that fruity-floral attributes drove consumer liking of a coffee flavored product. Therefore, one of ordinary skill in the art would not have been led to add a fruity-floral flavor ingredient to a coffee flavored product. It was only after we determined that fruity-floral drove consumer liking, and, in particular, linalool, that one would have any reason to add linalool to a coffee product.

19. In addition, even if one would have desired a fruity-floral attribute in coffee, one would not have known that linalool would have produced a desirable coffee product, let alone one which had a "linalool" flavor. Due to the coupling effect of flavor ingredients and the number of different coffee components and flavor ingredients in coffee, one of ordinary skill in the art would not have any reasonable expectation of success or would have been able to predict that linalool would drive consumer liking. Prior to the present invention, it was unpredictable that adding linalool to coffee would be desirable at all.

20. Moreover, the claimed amount of linalool does not produce what one would describe as a lemon flavor or in any way reflect the flavor of linalool. To the contrary, the coffee product resulting from adding the claimed amount of linalool would not be described by one of ordinary skill in the art as having a lemon flavor, although it does produce a fruity-floral attribute. Instead, the resulting coffee product would be described or characterized by a consumer as having a natural coffee flavor.

21. Furthermore, not only would one not have any reasonably apparent reason to add linalool to coffee, one would not have known how much to add in order to drive consumer liking. Even if one would have thought it desirable to have a fruity-floral attribute to coffee, one would not have been enabled to know how much linalool to add in order to drive consumer liking. For example, if one would have added the 100 parts per 1,000 of flavorant, as disclosed in Marmo et al., one would not have found that the added linalool drove consumer liking at all (see, e.g., present specification, paragraphs [0044]-[0047]. Therefore, even if one would have been led to add linalool, one would not have known to add a sufficient amount of linalool to produce a coffee product with improved consumer liking. Since the amount of linalool of Marmo et al. would not have been considered to improve liking, or change the flavor of the coffee product, one would not be led to increase that amount of linalool to have the claimed amounts. Again, it must be stressed that adding linalool to coffee does not produce a "linalool" flavored product. Therefore, one would not know how much linalool needs to be present in order to improve taste. In other words, the prior art fails to enable one to know to add linalool to coffee, let alone how much to add.

22. The amount of linalool added to a coffee product allows one to produce a coffee product which mimics the coffee attributes in high quality, more expensive coffee blends. Accordingly, linalool is added to a coffee product in order to produce a rich flavor which one would find in, or associate with, more expensive coffee blends. However, the linalool does not result in the coffee having what one would describe as a "flavored" coffee flavor. Instead, one would describe the coffee as having a traditional natural coffee flavor (see, e.g., present specification and the Kraft Research Report, Appendix F).

23. The undersigned declares further that all statements made herein of his knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

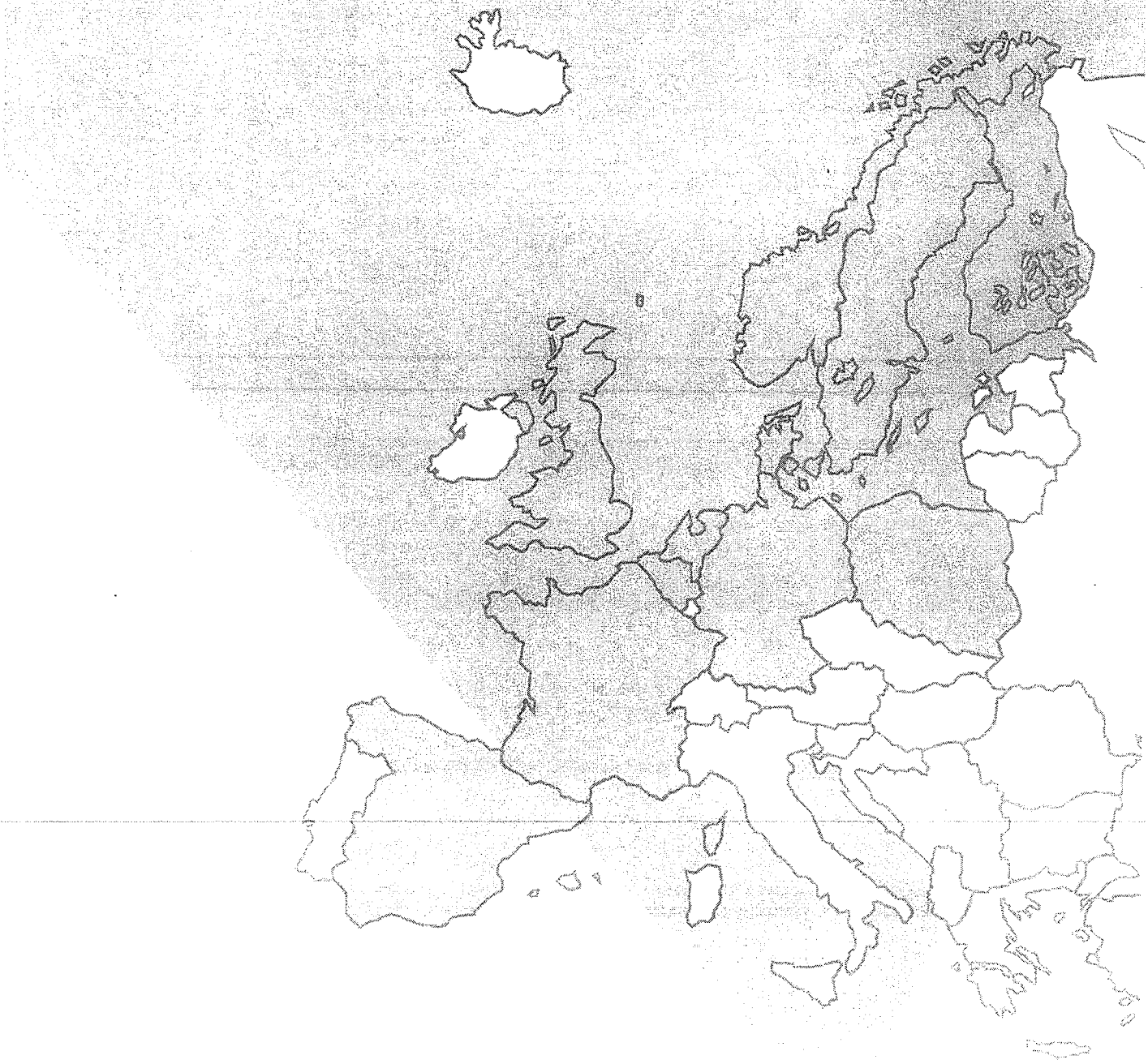
Signed this ____ day of _____ 2009.

_____
Mark Norton

APPENDIX A

A European Sensory and Consumer Study

A Case Study on Coffee



EUROPEAN SENSORY NETWORK

The attributes marked * in Table 5.1 indicate those which were developed by the ICO from extensive previous sensory work on coffee. These were provided to all panels as training aids, and many chose to use the ICO list as a basis for their vocabulary. This aspect clearly affects the occurrence of certain attributes by the panels (see Chapter 2 for details).

Table 5.1 Attributes used by more than one panel, followed by the number of attributes 'unique' to each of the eleven panels. * indicates an attribute from the ICO panel.

	PANEL											
Attribute	**DK**	**F1**	**F2**	**F3**	**GY**	**IO**	**NL**	**NY**	**PD**	**SN**	**UK**	**Total**
BITTERT*	1	1	1	1	1	1	1	1	1	1	1	11
BURNTF*	1	1	1		1	1	1	1	1	1	1	10
ACIDT*	1	1	1	1		1		1	1	1	1	9
ASTRGNF*		1	1	1	1	1		1	1	1	1	9
EARTHF*			1	1		1	1	1	1	1	1	8
CARAMF*	1		1	1		1		1	1		1	7
WOODF*	1			1		1		1	1	1	1	7
FLORALF*			1		1	1		1	1		1	6
FRUITF*			1	1		1		1	1		1	6
SWEETT*		1	1		1	1		1			1	6
BODYMF*		1				1		1	1	1		5
CHEMF*			1		1	1		1	1			5
CHOCF*						1	1	1	1		1	5
GRASSF*			1			1		1	1		1	5
RANCIDF*		1	1			1		1	1			5
RUBBERF*				1		1		1	1		1	5
SALTT*			1		1	1	1	1				5
MALTYF*			1		1	1		1				4
ROASTF			1	1			1				1	4
SMOKYF*				1		1				1	1	4
SPICYF*						1		1	1	1		4
TOBACOF*						1		1	1		1	4
ANIMALF*						1		1	1			3
ASHF*						1		1			1	3
BITTERAT					1		1			1		3
ENDUREAF				1	1		1					3
FLORALO		1			1		1					3
GREENF*					1	1	1					3
GREENO	1				1		1				1	3
LIQRICF				1			1				1	3
NUTF*						1		1				3
STRGNO				1	1		1					3

Table 5.1 - cont.

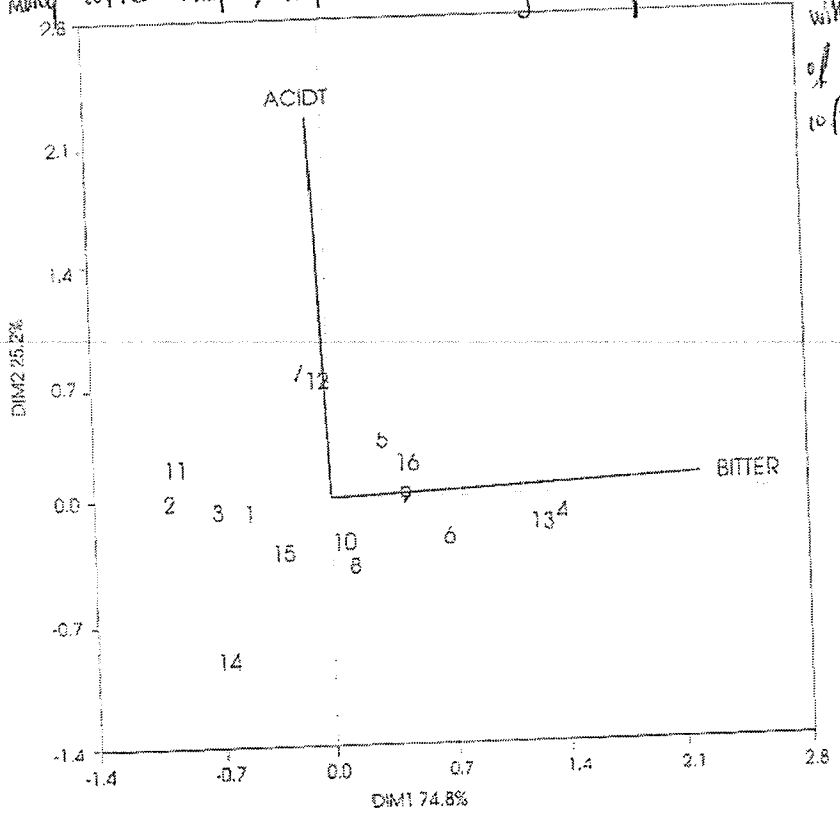
Attribute	PANEL DK	F1	F2	F3	GY	IO	NL	NY	PD	SN	UK	Total
RUBBERO	1	1					1					3
SOURF			1				1	1				3
SOURT*					1	1			1			3
SPICYO		1					1			1		3
THICKMF			1	1			1					3
ACIDAT										1	1	2
ACIDO	1									1		2
BITTERO					1					1		2
BURNTO	1									1		2
CARAMO	1						1					2
CEREALF*						1			1			2
CHICORF			1	1								2
CITRUSF*				1		1						2
COFFEEF	1			1								2
DRYMF					1		1					2
EARTHO		1								1		2
MEDICF*						1					1	2
METALF			1				1					2
MUSTYO					1		1					2
PEPPERF			1	1								2
ROASTO					1		1					2
ROTTENF*						1					1	2
SMOKYO							1			1		2
SOURAT					1		1					2
STRGNA			1	1								2
STRGNAF					1		1					2
STRGNF					1		1					2
SWEETAT				1	1							2
SWEETO					1		1					2
TARO							1			1		2
TOBACOAF			1								1	2
TOBACOO		1					1					2
WINEYF*						1		1				2
WOODO	1									1		2
Individual Attributes	**DK**	**F1**	**F2**	**F3**	**GY**	**IO**	**NL**	**NY**	**PD**	**SN**	**UK**	**Total**
	4	2	10	10	16	2	27	0	0	5	6	82

It was intended to start the construction of a European vocabulary from a fairly simplistic point of view, though to some this may be somewhat controversial. Attributes will be brought together to provide a fuller European sensory vocabulary based on results from this study.

The simple perspective, supported by intuitive reaction to the results of the cluster analysis, was to consider only the attributes bitter taste and acid taste. The rationale behind this was simply that two clusters highlighting both these attributes were apparent: Clusters 1 and 7 for bitter and Clusters 2 and 4 for acid. Undertaking PCA on these two attributes for the nine panels using both these attributes showed some interesting, though not totally unexpected, results. Figures 5.8 to 5.16 can be compared with Figures 4.1 to 4.11 to examine similarities in the sample structures. As can be seen, the overall structures are very similar. Thus, in general terms the coffee samples can largely be separated on these two basic tastes. However, there is some danger in reading too much into this simplistic view.

Bitter (extent of roast) and Acidity do a good job of describing the differences between many coffee samples, despite there being many different flavour attributes within the subtlety of an individual coffee (see next page)

Figure 5.8 Sample and attribute plot derived from covariance PCA on the two attributes acid and bitter for Denmark. Samples are labelled 1-16.



APPENDIX B

Lebensm Unters Forsch (1992) 195:239–245

Zeitschrift für
Lebensmittel-
Untersuchung
und-Forschung

Original paper

Potent odorants of the roasted powder and brew of Arabica coffee *

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Intensive Geruchsstoffe von Röstkaffee und Röstkaffeeaufguß aus Arabica-Kaffee

Zusammenfassung. Die Aromaextrakt-Verdünnungsanalyse (AEVA) von Röstkaffee ergab 13 wichtige Geruchsstoffe: 2-Methyl-3-furanthiol (I), 2-Furfurylthiol (II), Methional (III), 3-Mercapto-3-methylbutylformiat (IV), 3-Isopropyl-2-methoxypyrazin (V), 2-Ethyl-3,5-dimethylpyrazin (VI), 2,3-Diethyl-5-methylpyrazin (VII), 3-Isobutyl-2-methoxypyrazin (VIII), 3-Hydroxy-4,5-dimethyl-2(5H)-furanon (Sotolon, IX), 4-Vinylguajacol (XII) und (E)-β-Damascenon (XIII). Die vergleichende AEVA von Röstkaffee und daraus hergestelltem Aufguß zeigte im Aufguß eine Zunahme von III, IX, Vanillin und 4-Hydroxy-2,5-dimethyl-3(2H)-furanon und eine Abnahme von I, II, IV, V, VII und VIII.

Summary. Aroma extract dilution analysis (AEDA) revealed 13 compounds as important contributors to the aroma of roasted coffee (powder): 2-methyl-3-furanthiol (I), 2-furfurylthiol (II), methional (III), 3-mercapto-3-methylbutylformate (IV), 3-isopropyl-2-methoxypyrazine (V), 2-ethyl-3,5-dimethylpyrazine (VI), 2,3-diethyl-5-methylpyrazine (VII), 3-isobutyl-2-methoxypyrazine (VIII), 3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon, IX), 4-ethylguaiacol (X), 5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone (XI), 4-vinylguaiacol (XII), and (E)-β-damascenone (XIII). A comparative AEDA of the roasted powder and brew showed in the brew an increase of III, IX, vanillin and 4-hydroxy-2,5-dimethyl-3(2H)-furanone and a decrease of I, II, IV, V, VII, and VIII.

* Presented in part at the 14th International Conference on Science, San Francisco, USA (July 14–19, 1991)

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Introduction

The volatile fraction of roasted coffee has been analysed by many authors (reviews in [1, 2]), who identified more than 700 compounds [3] having a wide variety of functional groups. Attempts to determine those volatiles that actually contribute to the flavour of roasted coffee have been undertaken by Tressl [4] and, recently, by Holscher et al. [5, 6]. On the basis of the odour unit concept [7, 8], Tressl [4] suggested that 2-furfurylthiol, identified for the first time in coffee by Reichstein and Staudinger [9], was the most important odorant. In addition, he suggested further significant compounds for the flavour of coffee which were also confirmed in the present study. Recently, Holscher et al. [5, 6] using gas chromatography (GC) olfactometry of serial dilutions of the volatile fraction (aroma extract dilution analysis, AEDA [10]), established that some of the odorants suggested by Tressl [4] are indeed intensely involved in the coffee flavour and, in addition, they extended the number of key compounds responsible for the coffee flavour. The aim of the present study was to compare using AEDA the potent odorants of the roasted powder and of a brew prepared from this powder.

Materials and methods

Coffee. The Arabica coffee (*Coffea arabica*) from Columbia was supplied by Jacobs Suchard (Bremen, FRG). The coffee beans were medium roasted (3 min) using a Jetzone roaster. The particle size of the roasted and ground coffee was 300–500 µm. The coffee powder was packed in 500 g portions which were sealed under vacuum and stored at −35° C. Coffee powder (20 g) on a coffee-filter was extracted with hot water (80–100° C, 500 ml); the brew was immediately cooled in a water-bath at 12° C.

Chemicals. Pure compounds, corresponding to those in Table 2, were obtained commercially: nos. 2, 5, 6, 10, 11, 18, 20, 24, 25, 28, 30, and 2-hydroxy-3-methyl-2-cyclopenten-1-one (cyclotene) were from Aldrich (Steinheim, FRG); nos. 1, 7, 23, 32, 38 were from Merck (Darmstadt, FRG); nos. 31 and 34 were from Lancaster (Morecambe, UK); 2,3-pentandione (no. 3), hexamethyldisilazane

(HMDS) and diphenyltetramethyldisilazane (DPTMDS) were from Fluka (Neu-Ulm, FRG); methional (no. 8) was from Sigma (Deisenhofen, FRG) and no. 33 from IFF (Hilversum, the Netherlands). 3-Methyl-2-buten-1-thiol and (*E*)-β-damascenone were gifts from O.G. Vitzthum (Jacobs Suchard, Bremen, FRG) and G. Ohloff (Firmenich, Geneva, Switzerland), respectively. – The reagents for the synthesis of the reference substances were from Aldrich. – The solvents (analytical grade) were from Merck, except for Freon 11, which was from Aldrich. The solvents were purified by slow distillation on a Vigreux column (100 × 1 cm). Silica gel 60 (Merck) was treated with conc HCl and deactivated with approximately 7% (by mass) of water [11].

Syntheses

Bis(2-methyl-3-furyl)disulphide. This was obtained by oxidation of the corresponding thiol according to Evers et al. [12].

2-Hydroxy-3,5-dimethyl-2-cyclopenten-1-one. This was prepared following the procedure by Tonari et al. [13]. The 3-methyl-5-morpholinomethyl-2-cyclopenten-2-ol-1-one was reduced in a 72% yield with Zn in acetic acid at 80° C for 2 h. *2-Hydroxy-4-methyl-* and *2-hydroxy-3,4-dimethyl-2-cyclopenten-1-one.* Preparation of a mixture of these compounds was modeled on the work by Arnarp et al. [14] using 3-methylglutaric acid as the starting material. After esterification and condensation, the cyclic intermediate was thermally decarboxylated to the 4-methyl derivative. The 3,4-dimethyl derivative was prepared by methylation of the cyclic intermediate with CH_3I/OH^- and subsequent thermal decarboxylation. The mass spectrometry in electron impact mode [MS(EI)] of the two alkyl-2-cyclopenten-2-ol-1-ones agreed with literature data [14, 15].

Ethyldimethylthiazoles. These were prepared by the Hantzsch condensation of an appropiate α-halocarbonyl with a reactive thioamide [16, 17]. The MS(EI) spectra agreed with literature data.

5-Ethyl-2,4-dimethylthiazole. The starting material, 3-bromo-2-pentanone was prepared by adding bromine (2.5 g) dropwise at 45–50° C to a mixture of 2-pentanone (0.86 g), water (10 ml) and potassium chlorate (0.15 g). A heavier oil was formed, which was separated from the aqueous layer, washed with 5% sodium bicarbonate and water, dried over $CaCl_2$ and finally isolated by fractional distillation in a 53% yield.

A mixture of acetamide (0.3 g), phosphorous pentasulphide (0.225 g) and toluene (5 ml) in a 50-ml three-necked round-bottomed flash was heated in a water bath. When a black oily lower layer formed, 3-bromo-2-pentanone (0.4 g) was added dropwise at a rate sufficiently fast to maintain refluxing of the solvent. After completion of the addition the mixture was refluxed for 1 h, water (1 ml) and conc HCl (0.2 ml) were added and the mixture further heated for 1 h. The solvent was then distilled off through a short-pathway column, the residue was made basic with 50% aqeous NaOH and finally the compound was extracted with ether. The organic layer was dried over Na_2SO_4 and the ether removed by distillation at reduced pressure. Fractionation yielded (53%) of the target compound.

4-Ethyl-2,5-dimethylthiazole. This was prepared in a 62% yield following the same synthetic route as described above. The starting material for the synthesis of the intermediate 2-bromo-3-pentanone was 3-pentanone.

2-Ethyl-4,5-dimethylthiazole. This was prepared in a 46% yield with phosphorous pentasulphide, propionamide and 3-bromo-2-butanone, which was obtained from 2-butanone.

3-Mercapto-3-methyl-1-butanol. This compound (I, in Fig. 1) was synthesized according to Sweetman et al. [19] using some modifications. The starting material was ethyl 3,3-dimethylacrylic acid (III). The sulphur was introduced by reaction with benzylthiol to form the thioether (IV). The second step was the cleavage of the benzyl moiety with Na/NH_3 (Birch reduction) in order to produce the mercapto derivative (V), which was then reduced with $LiAlH_4$ yielding the desired thioalcohol (I).

3-Benzylmercapto-3-methylbutanoic acid ethylester (IV). A mixture of benzylthiol (0.15 mol) and ethyl 3,3-dimethylacrylate (0.15 mol) and piperidine (30 ml) was heated (150° C) under reflux for 2 h. The solution was cooled and acidified with diluted HCl (1 mol/L) to pH 2.0. The thioether (IV) was extracted with diethyl ether (3 × 50 ml) and the organic phase was washed with water (2 × 30 ml). After drying with $MgSO_4$ at 4° C (1 h), the solvent was removed by distillation. The residue was then fractionated under reduced pressure through a 25-cm Fisher column to afford pure material in a 91% yield: MS in chemical ionization mode [MS(CI)] = 253 ($M^+ + 1$).

3-Mercapto-3-methylbutanoic acid ethylester (V). A 500-ml three-necked flask was fitted with a dropping funnel and an inert-gas (N) inlet. Ammonia was condensed into the flask at –78° C and sodium pellets were added to the liquid until the solution became blue. The ester (IV; 100 mmol) was slowly dropped into the solution and then an additional amount of sodium pellets was added. The NH_3 was removed at room temperature under a stream of nitrogen during a 12-h period. The excess of sodium was destroyed by addition of methanol (30 ml). Compound V was enriched by extraction of: (i) the residue, which was acidified with diluted HCl (1 mol/L) to pH 2–3, with diethyl ether (2 × 150 ml); (ii) the ethereal solution obtained with aqueous sodium carbonate (10%, w/v, 3 × 150 ml) and after acidification (pH 2–3); (iii) the aqueous layer with diethyl ether (2 × 100 ml). The organic phase was washed with water and dried over Na_2SO_4 affording (V) in 50% yield: MS(CI) = 163 ($M^+ + 1$).

3-Mercapto-3-methyl-1-butanol (I). $LiAlH_4$ (1 mol/L, 50 mL) in diethyl ether was placed under an N_2 atmosphere in a 250-ml three-necked flask, fitted with a dropping funnel. A solution of 50 mmol (V) in dry diethyl ether (30 ml) was slowly dropped into the mixture, which was stirred. The reaction was completed by further stirring under reflux for 2 h. After cooling, the mixture was carefully treated with iced water until no gaseous H_2 was formed. H_2SO_4 (10%, v/v) was added to obtain a clear solution. The mercaptobutanol (I) was extracted with diethyl ether (2 × 100 ml) and purified by column chromatography on silica gel at 12° C. Elution with 50% (by vol.) diethyl ether in pentane gave pure (I) in 70% yield. MS(CI) = 121 (80, $M^+ + 1$), 87 (100, $M^+ + 1$-H_2S); MS(EI) = 120 (5, M^+), 86 (30), 75 (15), 71 (30), 69 (50), 68 (15), 56 (25), 55 (20), 43 (20), 41 (100), 39 (40).

3-Mercapto-3-methylbutylformate (II). The title compound (Fig. 1) was prepared by formylation of (I) according to Stoffelsma et al. [20]. The formylation reagent was prepared by addition of acetanhydride (30 mmol) to formic acid (30 mmol) at 45° C for 2 h in a screw-caped vial. After cooling with iced water, dry pyridine (3 mmol) was added and then 20 mmol of (I) was slowly dropped

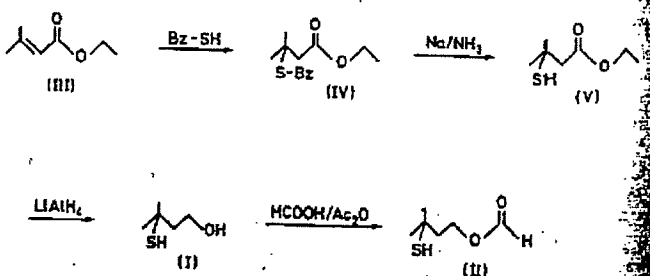

Fig. 1. Synthetic sequence for 3-mercapto-3-methyl-butanol (*I*) and its formic acid ester (*II*)

within 5 min into the reaction vial which was cooled with iced water. The mixture was heated for 4 h at 45–50° C to complete the formylation and after cooling to room temperature it was diluted with diethyl ether (50 ml), washed with $NaHCO_3$ (3%, w/v, 5 × 50 ml) and then with brine (2 × 50 ml) to neutrality and, finally, dried over Na_2SO_4 at 4° C for 1 h. After removal of the solvents the residue was fractionated under reduced pressure through a 25-cm Fisher column to afford pure material. MS(CI) = 149 (25, $M^+ + 1$), 115 (100, $M^+ + 1\text{-}H_2S$), 103 (40); MS(EI) = 148 (5, M^+), 102 (25), 87 (10), 75 (15), 69 (100), 41 (55).

Isolation of the volatiles

Roasted coffee powder. The roasted coffee powder (50 g) was placed in a 500 ml two-necked flask and diethyl ether/*n*-pentane (2+1, v/v, 150 ml) was added. The suspension was gently stirred for 10 min and then frozen for 30 min in liquid N_2. The flask was adapted to the apparatus described in [21, 22] and the volatiles were distilled off at 0.02 Pa for 3 h. The temperature of the water-bath was then increased to 50° C and the distillation continued for a further 2 h. The condensates of the first three cooling traps were combined, dried over Na_2SO_4 at 4° C and concentrated on a Vigreux column to 5 ml.

Coffee brew. The neutral components were extracted from the brew (500 ml) with CH_2Cl_2/Freon 11 (1+1, v/v, 500 ml) for 15 h using a rotation perforator (Normag, Hofheim am Taunus, Germany). The extract was concentrated on a Vigreux column (50 × 1 cm) to 120 ml and the volatile fraction was isolated by distillation under high vacuum as described above. The distillate was concentrated by microdistillation [23] to 2 mL. Thus, the concentration factor was the same (F = 10) in both samples, the roasted powder and brew.

Analytical methods

Column chromatography. The extracts of coffee powder (2 kg) and brew obtained by distillation in high vacuum were fractionated at 0–12° C on a water-cooled column (24 × 1 cm) packed with a slurry of silica gel 60 in pentane. The elution was performed with 50 ml pentane/diethyl ether (95:5, by vol., fraction A), 30 ml pentane/diethyl ether (75:25, by vol., fraction B), 30 ml pentane/diethyl ether (50:50, by vol., fraction C), and finally 100 ml diethyl ether (fraction D).

High performance liquid chromatography (HPLC). For the identification of compound no. 14 fraction B was chromatographed with the column and the apparatus described earlier [24]. Diethyl ether in pentane (3+97, v/v) was used as solvent mixture for separation into five subfractions that eluted in the ranges 4.0–6.5 ml (B I), 6.5–8.0 ml (B II), 8.0–10.5 ml (B III), 10.5–14.4 ml (B IV) and 14.4–20.0 ml (B V). Compound no. 14 was detected in the subfraction B IV. Fraction D was separated by HPLC using the conditions described in [25] in order to identify compound no. 17.

Capillary gas chromatography (HRGC). This was performed using the glass capillaries and fused-silica capillaries listed in Table 1. The glass capillaries were prepared according to Grob [26] using some modifications. The AR (alkali) glass capillary was leached with a solution of 20% HCl (10 h, 130° C), and washed with HCl (1 mol/L, 10 ml). The deionization process was followed by a dehydration step (230° C, 20 min, 2.7×10^3 Pa). The metal-free and dry inner surface was deactivated by persilylation (8 h, 400° C) using a solution of HMDS/DPTMDS/pentane (1+1+2, v/v/v). The capillary was subsequently rinsed with toluene, methanol and diethyl ether. The correctness of the procedure was checked by the ammonia test showing that about 60% of the capillary was filled with the solvent. After drying, the capillary was coated with the stationary phase (0.4% in methylene chloride) using the static method according to Bouche and Verzele [27].

The coated capillary was slowly heated (1° C/min) from 35° C to 250° C (10 h). The quality of the capillary was characterized by the "Grob-Test" [28], the separation efficiency ("Trennzahl") according to Kaiser [29] and the peak symmetry [30]. Some details are summarized in Table 1.

The samples were applied by the "on-column injection" technique. Retention indices (RI) were calculated, and the HRGC conditions were as described in [21]. Precolumns were renounced to avoid adsorption effects by the free silanol groups in the glass connector.

Stability of enoloxo compounds during HRGC. An aliquot (a1) of a stock solution of the compound in methanol was injected on each of the eight capillaries (Table 1). The peak area obtained was set equal to 100%. The stock solution was diluted with methanol as reported for AEDA [10] and aliquots (same volume as a1) of each diluted solution were analysed by HRGC on the capillaries listed in Table 1. Mean values were calculated of the areas of the peaks obtained for each couple of capillaries ("a" and "b" in Table 1) and then projected onto the peak area, which would give the amount dissolved in aliquot a1. These theoretical values were related to the 100% value and plotted versus the amounts of the substance analysed.

Table 1. Capillaries used for capillary gas chromatography (HRGC)

HRGC system	Stationary phase, film thickness (d_f)	Dimension and type of glass material	Trennzahl[a]		Peak symmetry[b]		
			$C_{10/11}$	$E_{10/11}$	ol	D	C_{11}
Ia	SE-54 $d_f = 0.3$ µm	25 m × 0.3 mm Soft glass	40	32	1.1	1.5	1.0
Ib	SE-54 $d_f = 0.3$ µm	30 m × 0.3 mm Soft glass	40	30	1.1	1.3	1.0
IIa	OV-1701[c] $d_f = 0.25$ µm	60 m × 0.3 mm Fused silica	55	40	1.0	1.0	1.0
IIb	OV-1701 $d_f = 0.3$ µm	30 m × 0.3 mm Soft glass	44	35	1.0	1.0	1.0
IIIa	Carbowax[d] $d_f = 0.25$ µm	30 m × 0.3 mm Fused silica	27	32	1.1	1.1	1.1
IIIb	Carbowax[d] $d_f = 0.25$ µm	30 m × 0.3 mm Fused silica	28	31	1.0	1.0	1.0
IVa	FFAP[c, e] $d_f = 0.25$ µm	30 m × 0.3 mm Fused silica	30	30	1.0	1.0	1.2
IVb	FFAP[c, e] $d_f = 0.25$ µm	30 m × 0.3 mm Fused silica	25	31	1.0	1.0	1.1

[a] Trennzahl (separation efficiency) according to Kaiser [29] using the GC conditions described in [26]. $C_{10/11}$, decane/undecane; C_{11}, methylester of decanoic acid and undecanoic acid

[b] Peak symmetry according to Meyer [30]. ol, 1,2-octanediol; D, 2,3-butandiol; C_{11}, methylester of undecanoic acid

[c] Fused silica (J & W) obtained from Macherey & Nagel (Hofheim, FRG)

[d] Fused silica Supelcowax 10 obtained from Supelco (Sulzbach, FRG)

[e] Free fatty acid phase

Mass spectrometry (MS)

MS analyses were performed on an MS 8230 (Finnigan MAT, Bremen, FRG) in tandem with the gas chromatography (GC) capillary columns described above using the same HRGC conditions. MS(EI) were generated at 70 eV and MS(CI) at 110 eV with isobutane as the reactant gas.

Gas chromatography/olfactometry (HRGC/O)

Aliquots of the volatile fractions were separated by HRGC[illegible] tailed in Table 2 and odorants were perceived at a sniffing por[illegible] 21]. The sensory significance of each odorant was evaluated an[illegible] pressed as the flavour dilution (FD) factor [10, 24]. O[illegible] thresholds in air were determined by HRGC/O [10, 21].

Table 2. Potent odorants (FD factor ≧16) of the roasted powder and brew of Arabica coffee

No.	Compound	Fraction[a]	Retention index on			Aroma quality[b]	FD factor[c]		Sensory significance established earlier[d]
			OV-1701	SE-54	FFAP		Powder	Brew	
1	2,3-Butandione[e] (diacetyl)	B	686	580	990	Buttery	16	32	[5]
2	3-Methylbutanal[e]	B	739	650	950	Malty	16	32	[4], [5]
3	2,3-Pentandione[e]	B	791	695	1060	Buttery	32	32	[4], [5]
4	3-Methyl-2-buten-1-thiol[f]	A/B	874	821		Amine-like	32	< 16	[5]
5	2-Methyl-3-furanthiol[f]	A	930	870		Meaty, boiled	128	< 16	[5]
6	2-Furfurylthiol[e]	A	1004	913	1440	Roasty (coffee-like)	256	64	[4], [5]
7	2-/3-Methylbutanoic acid[e]	D	1022	860		Sweaty	64	64	[5]
8	Methional[e]	C	1040	909	1455	Boiled potato-like	128	512	[5]
9	Unknown	D	1073		1365	Fruity	32	16	–
10	Trimethylthiazole[e]	B	1074	997	1370	Roasty, earthy	16	< 16	–
11	Trimethylpyrazine[e]	D	1078	1000	1395	Roasty, earthy	64	32	[5]
12	Unknown	C	1107	1055		Roasty, sulphury	128	32	–
13	3-Mercapto-3-methyl-1-butanol[e]	D	1127	972	1655	Meaty (broth)	32	64	[5]
14	3-Mercapto-3-methylbutylformate[e]	B	1138	1023	1517	Catty, roasty	2048	256	[5]
15	3-Isopropyl-2-methoxypyrazine[e]	B	1146	1097	1428	Earthy, roasty	128	32	[5]
16	5-Ethyl-2,4-dimethylthiazole[e]	D	1149	1078	1435	Earthy, roasty	32	16	–
17	2-Ethyl-3,5-dimethylpyrazine[e]	D	1154	1083	1453	Earthy, roasty	2048	1024	[4], [5]
18	Phenylacetaldehyde[e]	B/C	1178	1053	1635	Honey-like	64	32	[5]
19	Unknown	C/D	1185	1103		Roasty-earthy	128	128	–
20	Linalool[e]	C	1193	1102		Flowery	32	< 16	[5]
21	2,3-Diethyl-5-methylpyrazine[f]	C	1218	1155	1485	Earthy, roasty	512	128	–
22	2-Hydroxy-3,4-dimethyl-2-cyclopenten-1-one[e]	D	1226	1075	1840	Caramel-like	64	128	–
23	Guaiacol[e]	C	1228	1093	1850	Phenolic, burnt	32	16	[4], [5]
24	4-Hydroxy-2,5-dimethyl-3(2H)-furanone[e] (HDF)	D	1235	1065	2035	Caramel-like	16	256	[4], [5]
25	3-Isobutyl-2-methoxypyrazine[e]	B/C	1237	1186	1520	Earthy	512	128	[4], [5]
26	Unknown	C	1254	1184		Roasty, earthy	512	32	–
27	5-Methyl-5(H)-cyclopenta[b]pyrazine[e]	B	1260	1145		Roasty, sweet	32	< 16	[5]
28	(E)-2-Nonenal[e]	B	1275	1160		Fatty	64	< 16	[4], [5]
29	Unknown	D	1305		2090	Caramel-like	16	16	–
30	3-Hydroxy-4,5-dimethyl-2(5H)-furanone[e] (Sotolon)	D	1347	1107	2200	Seasoning-like	512	2048	–
31	4-Ethylguaiacol[e]	C	1424	1287	2032	Spicy	256	512	[5]
32	*p*-Anisaldehyde[e]	B/C	1431	1263	2030	Sweet, minty	32	< 16	–
33	5-Ethyl-3-hydroxy-4-methyl-2(5H)-furanone[e]	D	1433	1193	2270	Seasoning-like	512	1024	–
34	4-Vinylguaiacol[e]	C	1482	1323	2205	Spicy	512	512	[4], [5]
35	(E)-β-Damascenone[e]	B	1502	1395	1815	Honey-like, fruity	2048	64	[5]
36	Unknown	B/C	1620		2355	Amine-like	64	64	–
37	Bis(2-methyl-3-furyl)disulphide[f]	A	1640	1540	2150	Meaty, sweet	32	128	–
38	Vanillin[e]	D	1645	1410	>2500	Vanilla-like	32	512	–

[a] Fraction in which most of the compound appeared after enrichment by column chromatography

[b] Odour description assigned during aroma extract dilution analysis (AEDA)

[c] The flavour dilution (FD) factor of the compounds was evaluated using the capillaries given in brackets: no. 5 (SE-54), nos. 7, 13, 22–24, 30, 33, 38 (FFAP), the resting odorants (OV-1701)

[d] The sensory significance of the compound for the flavour of roasted coffee (powder) was reported by the quoted authors

[e] The compound was identified by comparing it with the reference substance on the basis of capillary gas chromatography (HRGC) on the capillaries presented in the table, the mass spectrum and the odour quality and threshold, which was perceived at the sniffing port

[f] The mass spectrometry signals of the substance were too weak for an interpretation; the compound was identified by comparing it with the reference substance on the basis of the resting criteria reported in footnote [e]

Results and discussion

Stability of enoloxo compounds during HRGC

Coffee contains 4-hydroxy-2,5-dimethyl-3(2H)-furanone (HDF) and other compounds having an enoloxo substructure [4, 5, 14]. HDF is partially degraded during analysis by HRGC [31], which would affect the results of AEDA. Therefore, in preliminary experiments, the yields of HDF and cyclotene, as examples for enoloxo compounds, after HRGC in relation to the amounts injected on GC capillaries coated with four stationary phases (Table 1) were determined. To calculate the yields of the diluted samples, the peak areas were projected on to that of the undiluted sample, which was set 100%. Figure 2 indicated that during HRGC on the relatively unpolar capillaries SE-54 and OV-1701 the yields of HDF and cyclotene decreased strongly with increasing dilution of the sample. HRGC on Carbowax improved the results but the highest yields were obtained on the capillary coated with FFAP. Consequently, this capillary was used for the AEDA of the enoloxo compounds.

Odorants of the roasted powder

AEDA of the coffee powder revealed 38 odorants with FD factors of 16 or higher. These compounds were enriched by column chromatography on silica gel as detailed in Table 2. Compounds nos. 14 and 17 were enriched further by HPLC of column fractions B and D, respectively. HRGC-MS analysis and HRGC/O of fractions A–D and the subfractions obtained from HPLC resulted in the identification of 28 odorants (Table 2). Four compounds (nos. 4, 5, 21 and 37) gave unclear MS signals. They were identified on the basis of the criteria reported in footnote "f" of Table 2. Six odorants, of which nos. 12, 19 and 26 appeared with higher FD factors, were not identified.

In the higher FD factor range 128 to 2048 (Table 2) the following 13 key compounds of coffee flavour were detected: 2-methyl-3-furanthiol (no. 5), 2-furfurylthiol (no. 6), methional (no. 8), 3-mercapto-3-methylbutylformate (no. 14), 3-isopropyl-2-methoxypyrazine (no. 15), 2-ethyl-3,5-dimethylpyrazine (no. 17), 2,3-diethyl-5-methylpyrazine (no. 21), 3-isobutyl-2-methoxypyrazine (no. 25), sotolon (no. 30), 4-ethylguaiacol (no. 31), 5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone (EHMF, no. 33), 4-vinylguaiacol (no. 34), and (*E*)-β-damascenone (no. 35). With the exception of sotolon, EHMF and 2,3-diethyl-5-methylpyrazine, these potent odorants were also identified by Holscher et al. [5, 6] in the fraction of the volatiles screened by an AEDA.

In the AEDA the authors [5, 6] diluted the extract 500-fold and found nine odorants at this level and identified eight of them. The potent odorants nos. 5, 6, 8, 14, 25, and 35 were among them as well as 2-/3-methylbutyric acid (no. 10) and HDF (no. 24), which showed relatively low FD factors in our study (Table 2). Holscher et al. [5, 6] reported two spicy odorants having high FD factors. On the basis of the RI data (2191 and 2257 on DB-Wax) reported [5, 6] we suggest that these odorants were identical with sotolon (no. 30) and EHMF (no. 33), respectively. Sotolon was also been found in sherry [32], cane sugar [33], fenugreek seeds [34], and aged sake [35], while EHMF has been detected in acid-hydrolysed vegetable proteins [36, 37].

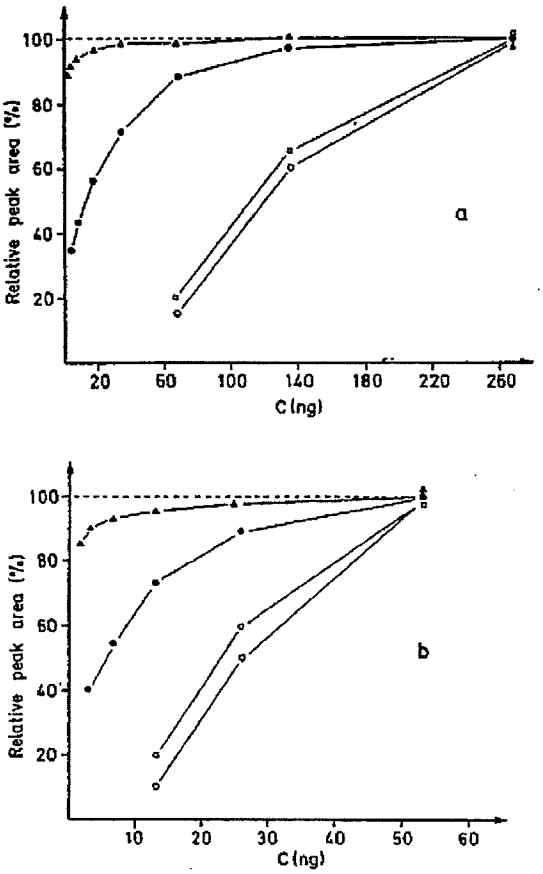

Fig. 2 a, b. Yields of 4-hydroxy-2,5-dimethyl-3(2H)-furanone (a) and cyclotene (b) after the capillary gas chromatography (HRGC) on capillaries SE-54 (○), OV-1701 (□), carbowax (●) and FFAP (△); C, compound; cf. Table 1

The conclusion of Tressl [4] that compounds nos. 2, 3, 6, 17, 23, 24, 25, 28, and 34 play an important role in coffee flavour was supported by AEDA, in particular for nos. 6, 17, 25, and 34, which showed higher FD factors (Table 2). Kahweofuran, which was also proposed as an impact compound of coffee flavour [4], was indeed identified (data not shown), but its very low FD factor suggested that it did not contribute to the coffee flavour.

Odorants of the brew

A brew was prepared from the powder of the Arabica coffee sample. Its odorants were extracted and evaluated by AEDA. As the FD factor of a compound is proportional to its concentration in the extract [10] the results listed in Table 2 suggested that, compared to the powder, methional (no. 8), HDF (no. 24), sotolon (no. 30) and

vanillin (no. 38) increased in the brew. On the other hand the thiols nos. 5, 6, 14 and the pyrazines nos. 15, 21, 25 decreased strongly. These changes in the concentrations of the odorants might be caused by a lower solubility in the brew, as was found for (*E*)-β-damascenone [22], and by degradation of the odorants by the hot water used for preparation of the brew. In addition, the FD factors of water-soluble odorants (e.g. diacetyl, 2,3-pentandione) might be reduced by a low extraction yield from the brew.

Odour thresholds

The odour thresholds in the air of some key compounds of the coffee flavour were evaluated (Table 3). The lowest threshold was found for 3-mercapto-3-methylbutylformate. It was 50-fold lower than the threshold of 2-furfurylthiol, the impact compound of the roasty odour note of the coffee powder. The threshold of 2-ethyl-3,5-dimethylpyrazine was in the same range as that of the 2-furfurylthiol. Together with (*E*)-β-damascenone, the threshold of which was four-fold lower, this pyrazine appeared with the highest FD factor in the extract of the coffee powder and was also important for the flavour of the brew. In the group of the enoloxo compounds, which contribute significantly to the flavour of the brew, the odour thresholds increased in the order 5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone, sotolon, 2-hydroxy-3,4-dimethyl-2-cyclopenten-1-one and HDF. Diacetyl and 2,3-pentandione were important odorants showing relatively high thresholds.

Table 3. Odour threshold of some volatiles identified in coffee (powder and brew)

Compound	Threshold (ng/L; air)	Capillary[a]
3-Mercapto-3-methylbutylformate	0.0002–0.0004	OV-1701
3-Isopropyl-2-methoxypyrazine	0.0005–0.001	OV-1701
2-Methyl-3-furanthiol	0.001 –0.002	SE-54
3-Isobutyl-2-methoxypyrazine	0.002 –0.004	OV-1701
5-Ethyl-3-hydroxy-4-methyl-2(5H)-furanone	0.002 –0.004	OV-1701
(E)-β-Damascenone	0.002 –0.004	OV-1701
2-Ethyl-3,5-dimethylpyrazine	0.007 –0.014[b]	OV-1701
2,3-Diethyl-5-methylpyrazine	0.009 –0.018[b]	OV-1701
2-Furfurylthiol	0.01 –0.02	OV-1701
Sotolon	0.01 –0.02	FFAP
4-Ethylguaiacol	0.01 –0.03	OV-1701
2-Hydroxy-3,4-dimethyl-2-cyclopenten-1-one	0.05 –0.1	FFAP
Methional	0.08 –0.16	OV-1701
4-Vinylguaiacol	0.4 –0.8	OV-1701
Trimethylthiazole	0.5 –1.0	OV-1701
Vanillin	0.6 –1.2	OV-1701
4-Hydroxy-2,5-dimethyl-3(2H)-furanone	0.5 –1.5	FFAP
3-Methylbutanal	2 –4	OV-1701
Diacetyl	10 –20	OV-1701
2,3-Pentadione	10 –20	OV-1701

[a] Capillary used for the determination of the odour thresholds by HRGC/olfactometry

[b] The thresholds were determined by C. Cerny (private communication)

Conclusions

The character impact odour compounds of the powder and brew of Arabica coffee are different. The contributions of thiol odorants (e.g. 3-mercapto-3-methylbutylformate, 2-furfurylthiol) and (*E*)-β-damascenone are stronger in the flavour of the powder than to that of the brew. The reverse effect was found for methional, sotolon, 4-hydroxy-2,5-dimethyl-3(2H)-furanone and vanillin. 2-Ethyl-3,5-dimethylpyrazine plays an important role in the flavours of both the powder and the brew.

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APPENDIX C

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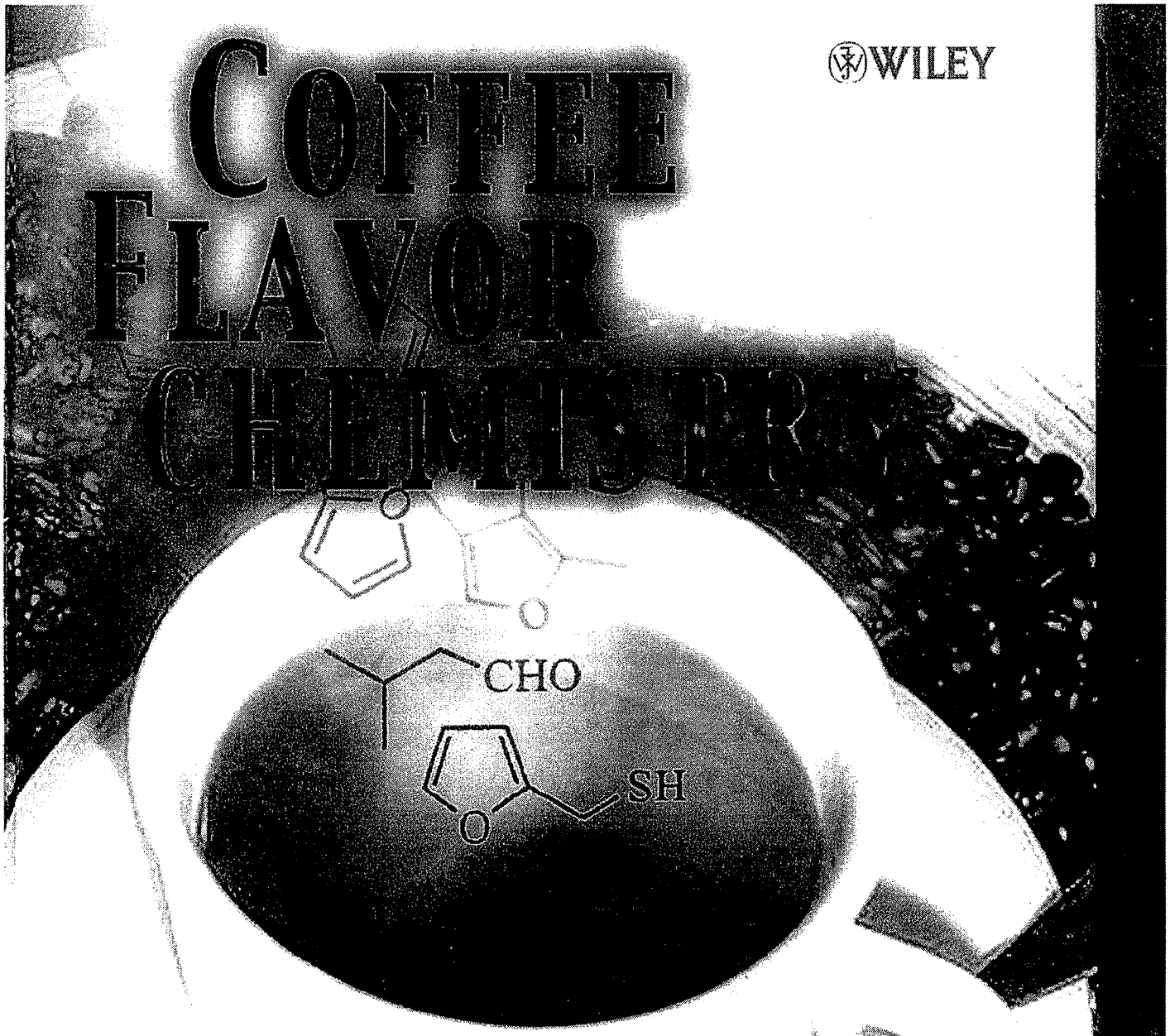
COFFEE FLAVOR CHEMISTRY



Ivon Flament

COFFEE FLAVOR CHEMISTRY

Ivon Flament
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With the collaboration of
Yvonne Thomas-Bessière

For the best part of two centuries investigators have tried with varying degrees of success to identify the compounds which give roasted coffee its characteristic aroma and taste. The analytical methods and the state of progress in chemistry at the end of the 19th century did not allow for the separation, isolation and identification of the multitude of trace chemicals which are present in roasted coffee. By 1900, scarcely a dozen compounds had been identified. Since the beginning of the sixties, with the advent of gas chromatography and mass spectrometry, the number of identifications has increased tremendously. To date, 850 compounds have been identified in the flavor of roasted coffee and 300 in the smell of green coffee.

In this work, the authors systematically review the non-volatile constituents of green coffee, including their structure, and discuss their important contribution as flavor precursors during the roasting process. They also trace the chronological discovery of the individual chemicals and critically examine the validity of their identification, highlighting the enormous progress which has been realized during the twentieth century and particularly in the last 40 years. For convenience, the constituents of green and roasted coffee have been distributed into chemical classes according to structure, systematic and empirical names, their CAS Registry Numbers and occasionally their FEMA classification. Comments are made on the origin or the formation during roasting of each individual compound.

Coffee Flavor Chemistry:

- contains an up-to-date list of almost 1400 original literature references;
- is the first book to provide a comprehensive overview of coffee flavor chemistry;
- critically discusses all of the identified and confirmed compounds in coffee;
- presents the major part of the book as a catalogue, for the benefit of the reader;
- includes information on structures, systematic and empirical names, identification, mechanism of formation, synthesis, detection threshold and organoleptic properties of each constituent where available;
- devotes a chapter to the flavor precursors, including the names and structures of the compounds with reference to the corresponding analytical work.

This book will be an invaluable reference for scientists – including analytical chemists and flavorists – in coffee companies, food industries, essential oils and flavor companies, pharmaceutical laboratories, food technology institutes, international and governmental regulation authorities and quality control laboratories.

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This alcohol has been identified as a degradation product of sotolone (**G.12**) in the presence of UV light (Martin *et al.*, 1990).

The flavor is fusel, solvent, estery, leafy (Chemisis, 1999)

(B.31) *(B.31)* **1-Octen-3-ol,** *oct-1-en-3-ol,* amyl vinyl carbinol, pentyl vinyl carbinol, 3-hydroxy-1-octene, Matsutake alcohol **[3391-86-4]** *FEMA 2805*; **(±)- [50999-79-6]; (*R*)-(-)- [3687-48-7]; (*S*)-(+)- [24587-53-9]**

Identified by Stoll *et al.* (1967) in roasted coffee, by Cros *et al.* (1980) (headspace analysis) and in green coffee by Gutmann *et al.* (1979). In green coffee, the concentration is 0.03 ppm for Holscher and Steinhart (1995) and the peak area in GC represents 0.60% of the volatiles for Cantergiani *et al.* (2001) (vacuum hydrodistillation).

It is one of the most intense flavor compounds formed by autoxidation of linoleic acid ($C_{18:2}$) (Ullrich and Grosch, 1987). As an aside, it is interesting to note that in mushrooms, an enzymic oxidative breakdown of linoleic acid gives the (*R*) isomer by the intermediate of 10(*S*)-hydroperoxy-8(*E*),12(*Z*)-octadienoic acid (Grosch and Wurzenberger, 1985).

These authors mention a mushroom aroma and give an odor threshold range of 2.3–5.3 ppb determined by high-resolution GC olfactometry. The (*S*)-isomer is described with a green, vegetable mouldy flavor (Chemisis, 1999) and the (*R*)-isomer with a green, mushroom meaty flavor (Chemisis, 1992).

(B.32) **7-Octen-4-ol,** *oct-7-en-4-ol,* 1-octen-5-ol **[53907-72-5]; (±)- [87830-31-7]**

Identified in green coffee by Gutmann *et al.* (1979) and found by Guyot *et al.* (1982, 1983) in healthy as well as 'stinking' green coffee.

It is characterized by a powerful, earthy note.

(B.33) **5-Hepten-2-ol,** 6-methyl-, *6-methylhept-5-en-2-ol* **[1569-60-4]; (±)- [4630-06-2]; (*R*)- [58917-27-4]; (*S*)- [58917-26-3]**

Identified by Cantergiani *et al.* (2001) in a green Mexican coffee (0.25% of the volatiles by GC). The corresponding ketone (**D.33**) has been identified but only in roasted coffee.

The racemic mixture has a green, fatty odor (Chemisis, 1981).

(B.34) *(B.34)* **3-Buten-2-ol, 2-methyl-,** *2-methylbut-3-en-2-ol,* 1,1-dimethyl-2-propenol, 1,1-dimethylallyl alcohol, dimethyl vinyl carbinol, 3-hydroxy-3-methyl-1-butene **[115-18-4]**

Identified by Silwar (1982). Silwar *et al.* (1987) give a concentration of 0.20–0.35 ppm in roasted coffee (steam distillation and distillation–extraction) and Ho *et al.* (1993) of 0.06 ppm in a Columbian coffee (headspace). Procida *et al.* (1997) found it only in a roasted arabica, disappearing upon prolonged roasting, and not in the various green arabicas and robustas that they examined. Cantergiani *et al.* (2001) identified it in a green Mexican coffee where it represents 0.13% of the volatiles by GC (after vacuum hydrodistillation).

The odor and flavor are described as solvent (Chemisis, 1972).

Linalool

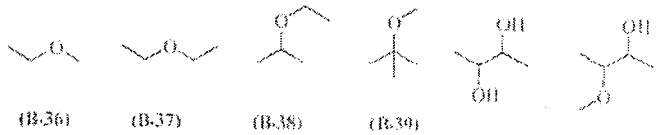
(B.35) *(B.35)* 1,6-Octadien-3-ol, 3,7-dimethyl-, *3,7-dimethylocta-1,6-dien-3-ol,* 2,6-dimethyl-2,7-octadien-6-ol, linalool **[78-70-6]** *FEMA 2635*; **(±)- [2256-4-99-4]**; licareol, **(*R*)-(-)- [126-91-0]**; coriandrol, **(*S*)-(+)- [126-90-9]**

Identified by Stoll *et al.* (1967), Stoffelsma *et al.* (1968), and Friedel *et al.* (1971) (IR, MS data given) in roasted coffee. Ho *et al.* (1993) gave a concentration of 0.73 ppm in a roasted Columbian coffee (head-

space). Linalool was found by Guyot *et al.* (1982, 1983) in 'stinking' green coffee, but not in a healthy variety. On the contrary Spadone *et al.* (1990) found it in green beans of a Puerto Rico 'Rio' and of a healthy variety. Holscher and Steinhart (1995) give a concentration of 0.175 ppm in green coffee (distillation–extraction at room temperature then at 70 °C). It represents 1.16% (GC) of the volatiles (vacuum hydrodistillation at room temperature) in the analysis of a green Mexican arabica by Cantergiani *et al.* (2001). Linalool has also been identified in red berries of an arabica (grown in a greenhouse), the amount decreasing with time with a concomitant increase of linalool oxides (see I.56, I.57, I.144 and I.145).

Linalool has a light and refreshing, floral-woody odor with a faintly citrusy note (Arctander, 1969). The flavor of the racemic is woody, floral, green, bergamot (Chemisis, 1991). The typical floral character can explain a somewhat undesirable note in disharmony with the notes of a roasted coffee. It is among the potent odorants of roasted powder of arabica coffee but not of the brew (Blank *et al.*, 1992b) and also of raw arabica coffee (Czerny and Grosch, 2000). Buttery *et al.* (1969b) give an odor threshold of 6 ppb in water, and Ahmed *et al.* (1978) of 5.3 ppb in water (confidence limits 1.9–15) with a flavor threshold in water of 3.8 ppb (confidence limits 1.4–10).

Diols and ethers



(B.36) (B.37) (B.38) (B.39) (B.40) (B.41)

(B.36) Ethane, methoxy-, *methoxyethane, ethyl methyl ether* **[540-67-0]**

Identified in headspace analysis of roasted coffee by Wang *et al.* (1983).

(B.37) **Ethane, 1,1'-oxybis-**, *ethoxyethane, diethyl ether*, ethylether, diethyl oxide **[60-29-7]**

Identified in four green arabicas (out of six) and in five green robustas (out of six) by Procida *et al.* (1997) who did not find it in a roasted arabica (headspace, GC/MS).

(B.38) Propane, 2-ethoxy-, *2-ethoxypropane, ethyl isopropyl ether* **[625-54-7]**

Identified by Ho *et al.* (1993) in a headspace of roasted Columbian coffee, with a concentration of 3.8 ppm.

(B.39) Propane, 2-methoxy-2-methyl-, *2-methoxy-2-methylpropane*, tert-*butyl methyl ether*, 1,1-dimethylethyl methyl ether **[1634-04-4]**

Identified in headspace analysis of roasted coffee by Wang *et al.* (1983).
Guyot *et al.* (1983) mention the presence of dimethoxycyclohexane, without any structural precision , in stinking as well as in healthy green beans.

(B.40) **2,3-Butanediol**, *butane-2,3-diol*, dimethylethyleneglycol, 2,3-butyleneglycol **[513-85-9]**; **(*R**,*R**)- [35007-63-7]**; **(2*R*,3*R*)- (or [*R*-(*R**,*R**)]-) (or levo, or threo, 2*R*,3*R*) [24347-58-8]**; **(2*S*,3*S*)- (or [*S*-(*R**,*R**)]-) (or dextro, or threo, 2*S*,3*S*) [19132-06-0]**; **(*R**,*S**)-** (or meso, or erythro, 2*R*,3*S*) **[5341-95-7]**

Identified by Vincent *et al.* (1976) in stinking green beans. It was noted as butane diol-2, in the text and in the summary, but with the presence of the corresponding diketone and hydroxyketone in the same

Methyl propanoate has a very diffusive, ethereal-rum-like odor, sweet and fruity of very poor tenacity (Arctander, 1967). The flavor is fruity, green, chemical (Chemisis, 1999). Ahmed *et al.* (1978) gave a probable odor threshold in water of 100 ppb and a probable flavor threshold in water of 58 ppb.

(F.26) **Propanoic acid, propyl ester,** *propyl propionate, propyl propanoate* **[106-36-5]** *FEMA 2958*

Identified in a green coffee headspace analysis by Merritt *et al.* (1970), only in a Columbian arabica and not in a Santos arabica and a robusta.

Fresh-ethereal, fruity-floral odor of very poor tenacity. The fruity notes being apple-pineapple-like, while the floral character is more of a general lift of sweetness (Arctander, 1967). The flavor is ethereal, fruity, rum, weak (Chemisis, 1998). The odor threshold given by Flath *et al.* (1967) was 57 ppb.

(F.27) 2-Propanone, 1-(1-oxopropoxy)-, *2-oxopropyl propionate, 2-oxopropyl propanoate,* hydroxyacetone propionate, acetol propionate, acetonyl propanoate **[72845-79-5]**

Identified by Bondarovich *et al.* (1967) in an 'aroma complex' of roasted coffee.

(F.28) Butanoic acid, methyl ester, *methyl butyrate, methyl butanoate* **[623-42-7]** *FEMA 2693*

Identified in roasted coffee but absent from green beans according to Merritt *et al.* (1970).

This ester has a very diffusive and penetrating, sweet-ethereal, fruity odor. In extreme dilution it is reminiscent of apple peel with a slightly fatty peach-like undertone (Arctander, 1967). It has a fruity, over-ripe, cheesy flavor (Chemisis, 1999). The probable threshold would be 43 ppb in water (confidence limits: 15–120 ppb) according to Ahmed *et al.* (1978) and the probable flavor threshold 59 ppb (confidence limits: 23–150).

(F.29) **Butanoic acid, ethyl ester,** *ethyl butyrate, ethyl butanoate* **[105-54-4]** *FEMA 2427*

Identified by Merritt *et al.* (1970) in a green coffee headspace of a Columbian arabica but not in a Santos arabica and a robusta coffee. It has also been found by Guyot *et al.* (1982, 1983) in stinking green coffee, and in a very low concentration in healthy beans of a Cameroon arabica.

Its pine-cone character can contribute to the defective off-flavor. Powerful, ethereal-fruity odor suggestive of banana and pineapple and very diffusive (Arctander, 1967). The flavor is fruity, fresh (Chemisis, 2000).

An odor threshold of 1 ppb is given by Flath *et al.* (1967). The flavor threshold was 0.45 ppm in water for Keith and Powers (1968) and 15 ppb for Siek *et al.* (1969). Ahmed *et al.* (1978) gave lower values: probable odor threshold in water 0.13 ppb (but with confidence limits 0.003–4.6) and flavor threshold of 0.13 ppb (with confidence limits 0.00008–2).

Butanoic acid, ethenyl ester, *vinyl butanoate* **[123-20-6]**

This was tentatively identified in headspace of roasted coffee by Wang *et al.* (1983).

(F.30) Pentanoic acid, methyl ester, *methyl pentanoate*, methyl valerate **[624-24-8]** *FEMA 2752*

Its presence in headspace of green coffee, but not of roasted beans, was mentioned by Merritt *et al.* (1970).

It is described as having a pungent-ethereal, green-fruity apple-like odor of poor tenacity (Arctander, 1967).

Of ~ 1150 compounds within this reference text very many have fruity or floral-type characters associated with them.

(F.31) **Pentanoic acid, ethyl ester,** *ethyl pentanoate*, ethyl valerate, ethyl valerianate **[539-82-2]** *FEMA 2462*

Identified by Merritt *et al.* (1970) in the headspace of green beans of a Columbian arabica (not in a Santos arabica or a robusta).

The odor is powerful and diffusive, ethereal-fruity, applelike with a remote resemblance to pineapple (Arctander, 1967). The flavor is described as juicy, winey, blueberry, tropical and apple (Leffingwell, 1999). An odor threshold of "ppb" was given by Pfaffmann (1959) and a flavor threshold of 8.4 ppb by Keith and Powers (1968).

(F.32) **Pentanoic acid, butyl ester,** *butyl pentanoate*, butyl valerate, butyl valerianate **[591-68-4]** *FEMA 2217*

Identified in green beans volatiles (simultaneous distillation–extraction and GC of the concentrate) by Spadone and Liardon (1987), only in Puerto Rico 'Rio' coffee and not in other 'Rio' and healthy beans.

The flavor is fruity, apricot-like (Chemisis, 1978).

(F.33) *(F.33)* **Hexanoic acid, methyl ester,** *methyl hexanoate*, methyl caproate **[106-70-7]** *FEMA 2708*

Identified by Merritt *et al.* (1970) in green and roasted beans.

It is described as having a powerful ethereal and diffusive, sweet odor of pineapple-apricot type (Arctander, 1967).

(F.34) **Hexanoic acid, ethyl ester,** *ethyl hexanoate*, ethyl caproate **[123-66-0]** *FEMA 2439*

Identified in green immature beans by Full *et al.* (2000), after electronic sorting of the beans followed by simultaneous distillation–extraction.

It is described as pineapple, banana, fruity. The odor threshold in water was 1 ppb according to Takeoka *et al.* (1995).

(F.35) Tetradecanoic acid, 1-methylethyl ester, *isopropyl tetradecanoate*, isopropyl myristate **[110-27-0]** *FEMA 3556*

Identified by Ramos *et al.* (1998) in headspace after solid-phase microextraction of a brewed arabica (compare with F.8 and F.19).

(F.36) *(F.36)* **Hexadecanoic acid, methyl ester,** *methyl palmitate, methyl hexadecanoate* **[112-39-0]**

Identified by Stoll *et al.* (1967) in a roasted coffee extract and by Cantergiani *et al.* (2001) in a green Mexican arabica extract (after vacuum hydrodistillation) where it represents 0.04% (GC).

(F.37) **Hexadecanoic acid, ethyl ester,** *ethyl palmitate, ethyl hexadecanoate* **[698-97-7]**

(F.38) **Hexadecanoic acid, butyl ester,** *butyl palmitate, butyl hexadecanoate* **[111-06-8]**

These have been found, like the lower homolog (see F.36), by Cantergiani *et al.* (2001) in a green Mexican coffee (respectively 0.12 and 0.05% of the extract in GC). F.37 had previously been tentatively identified by Spadone and Liardon (1988) in 'Rio' and healthy green beans.

The flavor of F.38 is described as fatty, waxy, mouthfeel (Chemisis, 1989).

APPENDIX D

In the last decade espresso coffee consumption has become a society phenomenon all over the world.

Espresso Coffee Second Edition, written by an international team of coffee experts, describes all the science and technology now available to obtain a perfect cup of espresso:

- Botany of both traditional and newly developed cultivars, with the new tools used in the identification of the required characteristics (biochemical ecology and molecular genetics).
- Agricultural conditions (environmental and cultural practices, and genetic aspects) most suited to the different production areas.
- Green coffee production including decaffeination, with the recent improvements in harvesting, processing and classification by physical, chemical and sensorial analysis.
- Storage and transportation best suited to avoid spoilage and quality loss.
- Physical and chemical changes produced by roasting, grinding and packaging, and the more and more sophisticated techniques now used to characterize them.
- Special percolation techniques are needed for the brewing of espresso coffee, where the quality and composition of the foam are as essential as those of the liquid, and the organoleptic characteristics of espresso partake of visual, gustatory, olfactory and somatic senses (mouthfeel and astringency).
- Finally, the physiological importance of coffee consumption, which, particularly in espresso, is more than just caffeine, is reviewed and evaluated.

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of a gas chromatograph is split to a conventional detector and a heated sniffing port, where trained people evaluate and record the sensory impression of individual compounds (Holscher *et al.*, 1990). Dilution techniques are used to determine the so-called flavour dilution (FD) factors. By a stepwise dilution of the aroma extract (1:1 by volume) followed by GC-O analysis the most important contributors can be smelled at the highest dilution, thus getting the highest FD factors. This technique, developed by Ullrich and Grosch (1987), is known as aroma extract dilution analysis (AEDA).The odour activity value (OAV) can be expressed as the concentration divided by its threshold only if the structure and the odour threshold of a substance are known. A method for the prediction of OAV from FD factors is reported as well as the precision and the optimal design of AEDA (Ferreira *et al.*, 2001).

4.4.4 Aroma impact compounds in roasted coffee

In arabica coffee the most important contributors to the aroma of roast and ground coffee are determined by the techniques mentioned above. 3-Mercapto-3-methylbutylformate (MMBF), 2-furfurylthiol, methional, β-damascenone as well as two pyrazines and furanones show the highest FD factors (Holscher *et al.*, 1992). A comparison of the results of the major research groups in coffee aroma is given in Table 4.5, together with the aroma impressions of the substance.

The absolute amounts of the aroma compounds shown in the last column differ by two orders of magnitude. The potency, especially that of the sulphur compounds, is demonstrated by their low concentrations. Because of a very low odour threshold (in air), only 130 µg/kg of MMBF are sufficient to generate the strongest odour impression, which is more than 10 000 times over its threshold of 0.003 ng/l of air (OAV of 37 000).

4.4.5 Effects on cup impression

Everybody who has ever had a really bad cup of coffee in direct comparison to a perfect one knows how big the difference can be. The perceived quality depends on objective criteria such as quality of green coffee, but also on subjective or cultural preferences like type of preparation.

Table 4.5 Aroma impact compounds in roasted coffee powder

Odorant	Odour impression	Holscher[1] (FD)	Grosch[2] (FD)	Schenker[3] (FD)	Mayer[4] (µg/kg)
Methanethiol	Putrid, cabbage-like	25		–	4500
2-Methylpropanal	Pungent, malty	100		–	24 000
2-Methylbutanal	Pungent, fermented	100		16–128	28 600
2,3-Butanedione	Buttery	200	–	256–1024	55 700
2,3-Pentanedione	Buttery	100	–	4–128	28 300
3-Methyl-2-buten-1-thiol	Animal-like, skunky	200	–	64–256	13
2-Methyl-3-furanthiol	Roasted meat-like	500	128	32	60
Mercaptopentanone	Sweaty, catty	100	–	–	–
2,3,5-Trimethylpyrazine	Roasty, musty	200	64	16–32	6000
2-Furfurylthiol	Roasty, coffee-like	500	256	1024	1350
2-Isopropyl-3-methoxy-pyrazine	Peasy	100	128	–	–
Acetic acid	Vinegar-like	100	–	–	–
Methional	Cooked potato	500	128	1024	148
2-Ethyl-3,5-dimethyl-pyrazine	Roasty, musty	200	2048	1024	55
(E)-2-Nonenal	Fatty	5	64	–	100
2-Vinyl-5-methyl-pyrazine	Roasty, musty	200	–		53
3-Mercapto-3-methyl-butylformate	Catty, roasted coffee-like	500	2048	1024	130
2-Isobutyl-3-methoxy-pyrazine	Paprika-like	500	512	4–64	84
5-Methyl-5H-6,7-dihydro-cyclopentapyrazine	Peanut-like	50	–		–
2-Phenylacetaldehyde	Honey-like	25	64	–	2500
3-Mercapto-3-methylbutanol	Soup-like	100	32	–	–
2/3-Methylbutanoic acid	Sweaty	500	64	1024	25 000
(E)-β-Damascenone	Honey-like, fruity	500	1024	16–128	258
Guaiacol	Phenolic, burnt	200	–	512–1024	3420
4-Ethylguaiacol	Clove-like	25	256	–	1780
4-Vinylguaiacol	Clove-like	200	512	256	45 100
Vanilline	Vanilla-like	–	32	–	4050

Peaks sorted by retention time on DB-wax column. Compounds with FD ≥ 32 are reported.
Sources: [1]Holscher et al. (1990); [2]Czerny and Grosch (2000); [3]Schenker *et al.* (2002), range according to roasting isothermal high/low temperature; [4]Mayer and Grosch (2001)

APPENDIX E

Lebensm.-Wiss. u.-Technol., 28, 310–313 (1995)

Analysis of Roasted Coffee Powders and Brews by Gas Chromatography-Olfactometry of Headspace Samples

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(Received November 21, 1994; accepted December 5, 1994)

The highly volatile, potent odorants of coffee samples were evaluated by gas chromatography-olfactometry of decreasing headspace samples (GCO-H). 2,3-Butanedione, 2,3-pentanedione, 3-methyl-2-butenthiol (I), methional, 2-furfurylthiol (II) and 3-mercapto-3-methylbutylformate (III) were the key odorants of both, the powders of Arabica and Robusta coffees. 2-Methyl-3-furanthiol (IV), 2,3-diethyl-5-methylpyrazine and an unknown compound were additional key odorants of the latter. An increase in the odour potencies of acetaldehyde, propanal, methylpropanal, 3-methylbutanal and dimethyltrisulphide as well as a decrease in the odour potencies of the thiols I to IV in the brews were the major differences with regard to the powders.

Introduction

Odorants contributing to the smell of freshly roasted coffee beans have been screened by aroma extract dilution analysis (AEDA) and then identified (1,2). However, AEDA is limited to odorants boiling higher than the solvent used for the extraction and dilution steps. Furthermore, odorants boiling in the same range as the extraction solvent are partially lost during the concentration of the extract by distilling off the solvent. To overcome these limitations, AEDA was completed by gas chromatography-olfactometry of headspace samples (GCO-H) (3,4).

The highly volatile odorants of freshly roasted coffee beans have been screened by GCO-H (3), and, by analogy with AEDA (5) the results have been expressed as flavour dilution (FD) factors. The FD-factor for a compound is a measure for its odour potency and is defined as ratio of its concentration in the largest headspace sample to its concentration in the smallest one in which odour was detected by GCO (3). In the headspace of coffee 12 odorants were identified in the largest sample of 250 µL (3). Methanethiol and 2-methylpropanal showed the highest FD-factor of 25; 2,3-butanedione, 2,3-pentanedione, 3-methylbutanal and 2-furfurylthiol followed with an FD-factor of 5.

Headspace samples often contain odorants in such low concentration levels that they give no detector response. In the case of coffee, 2-furfurylthiol, 3-methyl-2-butenthiol and methional are examples which are only detectable by sniffing of the GC effluent (3). However, as discussed recently (4), most of these odorants can be identified in headspace samples on the basis of the results obtained by a preceding AEDA.

In the present study, GCO-H was applied to compare the highly volatile odorants of the powders and brews prepared from roasted Arabica and Robusta coffees. In addition, a brew obtained from a soluble coffee powder was included in the comparison.

Materials and Methods

The Arabica coffee (*Coffea arabica*) from Columbia and the Robusta coffee (*Coffea canephora var. robusta*) from Indonesia were medium roasted and then ground (particle size: 300–500 µm). The coffee powders were packed in 1 kg portions which were sealed under vacuum and stored at –35°C until use. Hot water (1.1 L, ca. 95 °C) was poured on the coffee powder (54 g) in a filter (Kaffee-Filterpapier Nr. 4, Plus Warenhandelsgesellschaft, Hamm, Germany) yielding 1 L of the coffee brew. A soluble coffee powder was purchased from a local market; it (16 g) was dissolved in 1 L of hot water (ca. 95 °C).

Pure compounds, corresponding to those in **Table 1** were obtained commercially from the sources (in brackets) : nos. 1, 3 to 8, 11 to 13, 22, 24, 26 (Aldrich, Steinheim, Germany), no. 2 (Fluka, Neu-Ulm, Germany), no. 16 (Haarmann & Reimer, Holzminden, Germany), nos. 28 and 29 (Lancaster, Eastgate, UK). The following reference substances were synthesized according to the literature: 3-methyl-2-butenthiol (6),

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Table 1 GCO-H of roasted coffee powders and brews

No. of compound[a]	Odour description[b]	RI on RTX-5	FD-factor[c]				
			Powder		Brew		
			Arabica	Robusta	Arabica	Robusta	Soluble coffee
1 Acetaldehyde[d]	Fruity, pungent	<400	25	25	125	62.5	12.5
2 Methanethiol[d]	Putrid, cabbage-like, sulphurous	<400	5	12.5	1	1	n.d.
3 Propanal	Fruity	≈450	5	5	25	25	25
4 Methylpropanal[d]	Fruity, malty	≈550	5	5	25	25	25
5 2,3-Butanedione[d]	Buttery	580	62.5	125	125	125	62.5
6 3-Methylbutanal[d]	Malty	653	12.5	25	62.5	62.5	25
7 2-Methylbutanal[d]	Malty	662	5	12.5	25	25	12.5
8 2,3-Pentanedione[d]	Buttery	697	125	125	125	62.5	62.5
9 3-Methyl 2-butenthiol[e]	Foxy, skunky	822	62.5	62.5	5	5	1
10 Unknown	Meaty, roasty	839	n.d.	5	n.d.	n.d.	n.d.
11 2-Methyl-3-furanthiol[e]	Boiled meat-like	870	25	125	5	5	n.d.
12 Methional[e]	Boiled potato-like	906	62.5	62.5	25	25	5
13 2-Furfurylthiol[e]	Roasty	911	62.5	125	12.5	12.5	n.d.
14 Unknown	Roasty	939	n.d.	5	n.d.	n.d.	n.d.
15 Dimethyltrisulphide[e]	Cabbage-like	970	n.d.	1	12.5	25	n.d.
16 1-Octen-3-one[e]	Mushroom-like	978	n.d.	n.d.	5	1	n.d.
17 Unknown	Roasty	986	1	25	n.d.	1	n.d.
18 Unknown	Musty, earthy	1002	n.d.	n.d.	5	5	n.d.
19 Unknown	Earthy, roasty	1012	n.d.	n.d.	5	5	n.d.
20 3-Mercapto-3-methylbutylformate[e]	Catty, roasty	1022	62.5	62.5	1	1	n.d.
21 2-Ethyl-3,5-dimethylpyrazine[e]	Earthy, roasty	1086	25	62.5	25	62.5	25
22 Guaiacol[e]	Phenolic, burnt	1092	12.5	25	25	12.5	5
23 Unknown	Earthy, roasty	1107	25	125	25	25	5
24 2,3-Diethyl-5-methylpyrazine[e]	Earthy	1155	25	125	25	125	12.5
25 Unknown	Earthy	1182	12.5	62.5	1	25	12.5
26 2-Isobutyl-3-methoxypyrazine[e]	Green, earthy	1186	25	1	125	1	n.d.
27 Unknown	Roasty, sulphurous	1225	1	5	1	5	n.d.
28 4-Ethylguaiacol[e]	Phenolic, spicy	1282	n.d.	n.d.	n.d.	5	n.d.
29 4-Vinylguaiacol[e]	Phenolic, spicy	1317	n.d.	n.d.	5	25	n.d.
30 (E)-β-Damascenone[e]	Honey-like, fruity	1400	5	5	n.d.	n.d.	n.d.

[a] The compounds which appeared in one of the coffee samples with an FD-factor of at least five are compared.
[b] Odour description assigned during GCO-H.
[c] The relationship between FD-factor and headspace volume is given in **Table 2.**
[d] The compound was identified by comparing it with the reference substance using the following criteria: retention index (RI) on the capillary RTX-5, the mass spectrum and the odour quality perceived at the sniffing port.
[e] The compound was identified by comparing it with the reference substance on the basis of the RI on capillary RTX-5 and the odour quality perceived during GCO-H.
n.d.: not detectable in the highest headspace volume of 25mL **(Table 2).**

dimethyltrisulphide (7), 3-mercapto-3-methylbutylformate (2), 2-ethyl-3,5-dimethylpyrazine (8), (E)-ß-damascenone(9).

Gas chromatography-olfactometry

GCO-H was performed with a CP-9001 gas chromatograph connected to the purge and trap system TCT/PTI 4001 (Chrompack, Frankfurt, Germany). The TCT/PTI 4001 system was programmed and controlled via the keyboard of the gas chromatograph. The empty glass tube in the desorption heating block of the purge and trap facility was deactivated by treatment with a mixture of 1,3-diphenyl-1,1,3,3-tetramethyldisilazane, hexamethyldisilazane and pentane (1 : 1 : 2, v/v/v) (H. Guth, personal communication). The gas chromatograph was equipped with a cooling system for the oven (Chrompack) and with a RTX-5 (crosslinked SE-54) fused silica capillary (30 m × 0.52 mm, film thickness 1.5 μm; Amchro, Sulzbach/Taunus, Germany). At the exit of the capillary, the effluent was split (1 + 1, v/v) into a FID and a sniffing port by using deactivated fused silica capillaries (30 cm × 0.10 mm). The FID and the sniffing port were held at a temperature of 200 °C. Nitrogen (20 mL/min) was used as make-up gas for the FID. After each run the purge system was automatically cleaned (clean-up flow: 50 mL helium clean-up temperature: 275 °C).

Coffee powder (100 mg) was put into a vessel (volume: 250 mL), sealed with a septum and then held at room temperature for 30 min. Coffee brew (10 mL) was put into a vessel (volume: 100 mL), sealed with a septum and then held in a water bath at 70 °C for 30 min.

The headspace volumes given in **Table 2** were drawn by a gas-tight syringe and then injected with a velocity of about 20 mL/min into the purge system which operated in the desorption mode for 10 min at a temperature of

Table 2 Injected headspaces volumes and corresponding FD-factors

Headspace volume (mL)	25	5	2	1	0.4	0.2
FD-factor	1	5	12.5	25	62.5	125

280 °C. The carrier gas helium (flow: 20 mL/min) swept the headspace sample into the trap (20 cm × 0.53 mm fused silica capillary coated with CP-sil 8CP, film thickness 5 μm) which was cooled with liquid nitrogen at –110 °C. To start the GC run, the trap was heated up very rapidly to 250 °C. This temperature was held for 1 min, and the sample was flushed by the helium (flow rate: 8 mL/min) onto the RTX-5 capillary. The temperature of the oven was held at 0 °C for 2 min, then raised at a rate of 6 °C/min to 50 °C, held for 2 min and then raised at a rate of 8 °C/min to 250 °C.

Headspace — GC/MS

The apparatus used for GCO-H was modified. The RTX-5 capillary was replaced by a DB-5 fused silica capillary (30 m × 0.32 mm, film thickness 0.25 μm; J & W. Scientific, Folsom, U.S.A.). The exit of the capillary was coupled with the mass spectrometer Incos XL (Finnigan, Bremen, Germany). The flow of the carrier gas helium was 2 mL/min, and the same temperature program as reported under Headspace-GC/O was used. Headspace samples of 20 mL were analysed as reported above. Mass spectra were generated in the electron impact mode (MS/EI) at 70 eV.

Results and Discussion

The results of GCO-H of the coffee samples are summarized in **Table 1**. Twenty odorants (nos. 1 to 9, 11 to 13, 20 to 26, 30) showing FD-factors of 5 and higher were perceived in the headspace of Arabica coffee (powder). Odorants nos. 1 to 8 were identified by comparing the RI value, the mass spectrum and the odour quality with the corresponding properties of the reference substance. No mass spectrum was obtained in the cases of nos. 9, 11 to 13, 20 to 22, 24, 26 and 30. These odorants have been identified in the preceding AEDA (1,2) and most of them have been also quantified (10). Consequently, it was easy to identify them during GCO-H by comparing their RI values and their odour qualities with the corresponding properties of the reference substances. Only the earthy, roasty compounds nos. 23 and 25 remained unidentified.

There was a good agreement in the highly volatile odorants with those reported by Holscher and Steinhart (3) for Arabica coffee (powder). With exception of hydrogen sulphide and dimethylsulphide, all of the odorants (nos. 1, 2, 4 to 9, 12, 13 in **Table 1**) sniffed by the authors (3) were also perceived in this study. In addition, the thiols 11 and 20 were identified showing high FD-factors in the headspace of both coffee powders (**Table 1**). According to the results of preceding experiments (Guth and Grosch, unpublished results), these odorants are only detectable by GCO-H when the empty glass tube in the desorption block is deactivated (cf. **Materials and Methods**).

With exception of no. 26, all odorants with an FD-factor of at least five in the headspace of the Arabica coffee occurred also in that of the Robusta coffee (powder), but the latter contained three further odorants (nos. 10, 14, 17) of unknown chemical structure.

On the basis of their high FD-factors, 2,3-butanedione, 2,3-pentanedione, 3-methyl-2-butenthiol, methional, 2-furfurylthiol and 3-mercapto-3-methylbutylformate were the key, highly volatile odorants of both powders. The FD-factors of these and of the odorants nos. 1 to 4, 6, 7, 21, 22 and 30 were identical in both coffee samples, as they differed, at the most, by only one dilution step, which is within the limit of error of the GCO-H. Only quantitative measurements as performed in (10) could make clear whether the concentration levels of these odorants are different in the coffee samples. In contrast, clear differences in the odour potencies were found for 2-methyl-3-furanthiol, 2,3-diethyl-5-methylpyrazine and the unknown no. 23, which all belong only to the key odorants of the Robusta coffee powder on the basis of their high FD-factors (**Table 1**).

Brews were prepared from both coffee powders and held at 70°C until the headspace samples were drawn and analysed by GCO-H. This was performed very carefully, as the high water pressure at 70°C might damage the stationary phases of the GC capillaries. The results listed in **Table 1** indicate that the powders and the brews agreed in compounds nos. 5 and 8 as major odorants. The well-known, strong difference between the overall odours of the powders and the brews was mainly due to a decrease of the FD-factors of the thiols nos. 2, 9, 11, 13 and 20 as well as to an increase in the FD-factors of acetaldehyde, propanal, methylpropanal, 3-methylbutanal and dimethyltrisulphide in the brews. Furthermore, the guaiacols 28 and 29 were detectable in the brew of Robusta coffee, and 29 also in that of the Arabica coffee.

A brew prepared from a soluble coffee powder was also analysed by GCO-H. The results in **Table 1** reveal that the potency of the major odorants nos. 3 to 8, 21, 22, 24 and 25 agreed with that of the corresponding odorants of either the Arabica or the Robusta coffee brew or of both. On the other hand, the brew prepared from the soluble coffee powder was found to lack acetaldehyde, the thiols nos. 9, 11, 13, methional, unknown no. 23 and the guaiacols 28 and 29 compared to the brews prepared from the Arabica and the Robusta coffee samples.

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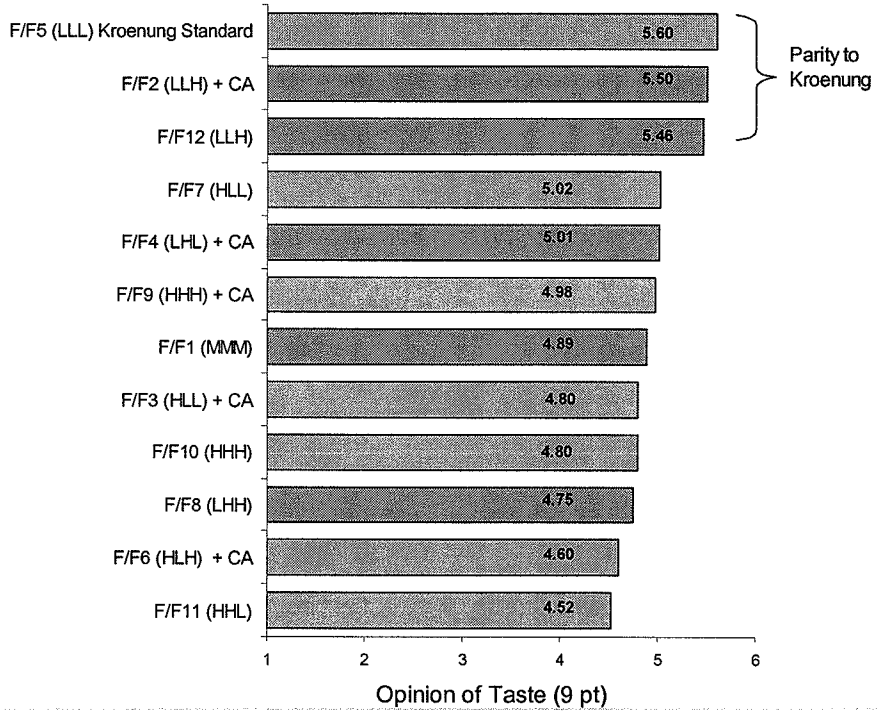
APPENDIX F

3.2 Enhanced Roast Coffee (outside standard of identity)

In this case, 11 prototypes were produced by adding combinations of 3 chemicals (linalool, ß-damascanone and phenyelthanol) into the already brewed coffee following an experimental design. Base used for the addition of chemicals was standard Jacobs Kroenung that was used as reference as well.

When results are analysed for all consumers, only 2 products scored parity with Kroenung although numerically inferior (Chart 6). All the rest scored significantly lower for this category of consumers.

Chart 6: Overall liking for all consumers



Products codes: (linalool, ß-damascanone and phenyelthanol) L= None, H = Addition of the chemical

Chart 7 reflects the results when the analysis is focused in fruity/floral likers (40%, identified through the classification set referred previously). In this case, 4 products scored numerically better than the reference, with one of them being significantly superior. This superiority is driven by the presence of linalool in the Kroenung base and it can be seen in most products with the high scores. The prototypes with linalool scoring lower tend to be those having additional acidity (citric acid). This confirms the sensory assessment of the acidity-fruitiness axis explained in the previous section where fruity/floral likers are described as fruity likers and acidity dislikers. On the other side, Chart 8 shows the tolerance of the fruity/floral dislikers to acidity and their dislike for fruitiness with products with linalool scoring low and products with added acidity spreaded across the scores.

Chart 7: Overall liking for fruity/floral likers (40% all consumers)

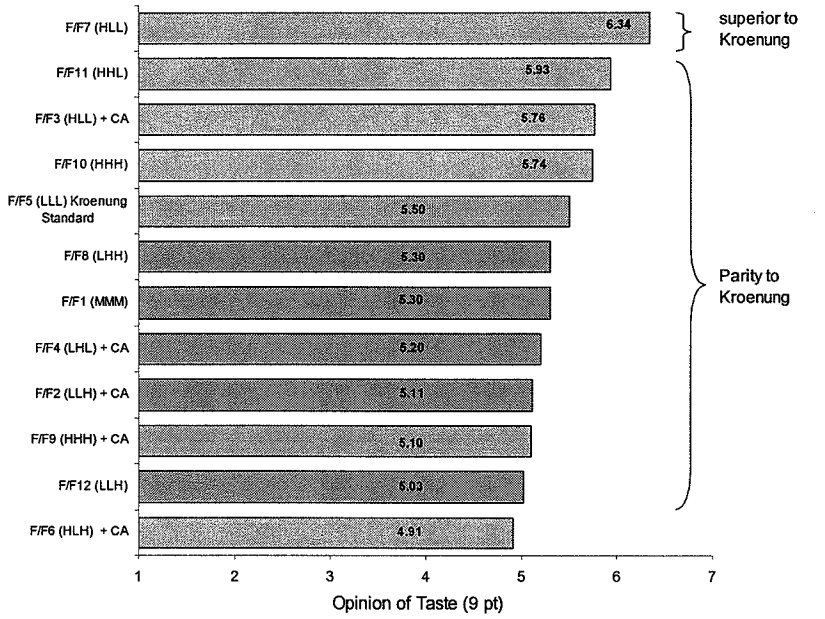
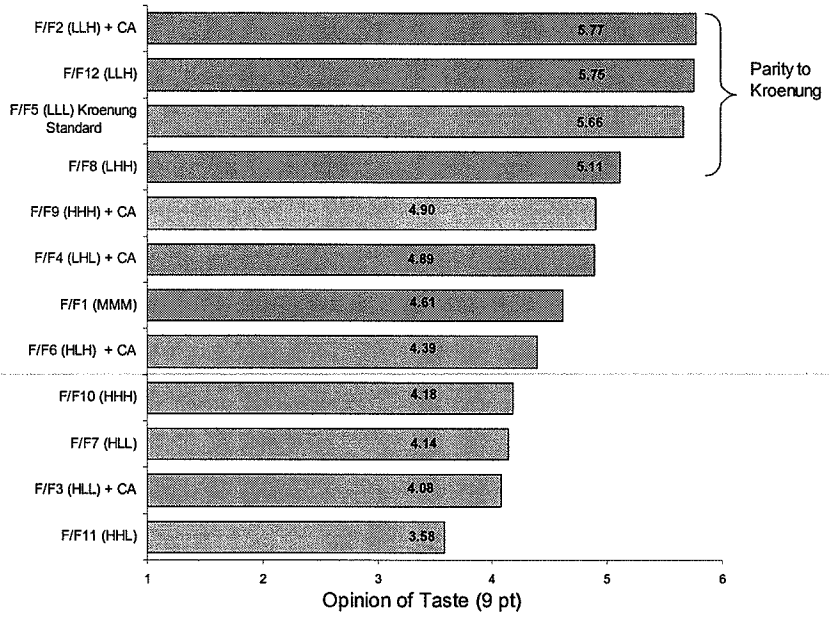


Chart 8: Overall liking for fruity/floral acceptors/dislikers (60% all consumers)

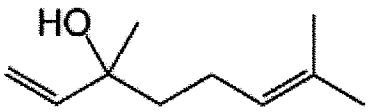


APPENDIX G

Linalool

From Wikipedia, the free encyclopedia

Linalool (IPA: /lɪˈnæloʊɒl/) is a naturally-occurring terpene alcohol chemical found in many flowers and spice plants with many commercial applications, the majority of which are based on its pleasant scent (floral, with a touch of spiciness). It has other names such as β-linalool, linalyl alcohol, linaloyl oxide, *p*-linalool, allo-ocimenol and 2,6-dimethyl-2,7-octadien-6-ol.

Linalool	
	
IUPAC name	[show]
Identifiers	
CAS number	78-70-6
PubChem	6549
SMILES	[show]
InChI	[show]
Properties	
Molecular formula	$C_{10}H_{18}O$
Molar mass	154.25 g/mol
Density	0.858 – 0.868 g/cm^3
Melting point	< 20 °C
Boiling point	198 – 199 °C
Solubility in water	1.589 g/l
Hazards	
Flash point	55 °C
Except where noted otherwise, data are given for materials in their standard state (at 25 °C, 100 kPa) Infobox references	

Contents

In nature

Over 200 species of plants produce linalool, mainly from the families Lamiaceae (mints, scented herbs), Lauraceae (laurels, cinnamon, rosewood) and Rutaceae (citrus fruits), but also birch trees and other plants, from tropical to boreal climate zones. It was also found in some fungi.

Enantiomers

Linalool has a chiral center at C_3 and therefore two stereoisomers: **licareol** is (R)-(+)-linalool with CAS No. 126–90–9 (PubChem 67179) and **coriandrol** is (S)-(–)-linalool with CAS No. 126–91–0 (PubChem 13562).

Both enantiomeric forms are found in nature: S-linalool is found, for example, as a major constituent of the essential oils of coriander (*Coriandrum sativum* L. family Apiaceae) seed, palmarosa [*Cymbopogon martinii* var martinii (Roxb.) Wats., family Poaceae], and sweet orange (*Citrus sinensis* Osbeck, family Rutaceae) flowers. R-linalool is present in lavender (*Lavandula officinalis* Chaix, family Lamiaceae), laurel (*Laurus nobilis*, family Lauraceae), and sweet basil (*Ocimum basilicum*, family Lamiaceae), among others.

Each enantiomer evokes different neural responses **in humans**, and therefore are anthropophilically classified as possessing distinct scents. 3S-(+)-linalool is perceived as sweet, floral, petitgrain-like (odour threshold 7.4 ppb) and the 3R-form as more woody and lavender-like (odour threshold 0.8 ppb)

Biosynthesis

In higher plants linalool as other monoterpenoids is produced from isopentenyl pyrophosphate via the universal isoprenoid intermediate geranyl pyrophosphate, through a class of membrane-bound enzymes named monoterpene synthases. One of these, linalool synthase (LIS), has been reported to produce (S)-linalool in several floral tissues.

Uses

In addition to its use as a scent in domestic products such as soap, detergent, shampoo, and lotion, linalool is also used as a chemical intermediate. One common downstream product of linalool is Vitamin E. Linalool, is also used by pest professionals as a flea, and cockroach insecticide

Safety information

Linalool should be avoided by people with perfume allergy[1].

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Notes

1. ^ Survey and health assessment of chemical substances in massage oils (http://www2.mst.dk/udgiv/publications/2006/87-7052-278-2/html/kap07_eng.htm)

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External links

- Comprehensive data sheet (http://www.inchem.org/documents/sids/sids/78706.pdf)
- Record (http://householdproducts.nlm.nih.gov/cgi-bin/household/brands?tbl=chem&id=2771) in the Household Products Database of NLM

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Categories: Terpenes and terpenoids | Flavors | Alcohols | Plant toxin insecticides